The value of a rapid cytological diagnosis in patients with breast lesions

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Carpe diem

aan mijn jongens Marcel en Pieter

aan mijn ouders

Contents

Chapter 1

Introduction and outline of thesis

Carcinoma of the breast is world wide a frequent problem. The frequency in industrialized countries is, however, much higher than in less developed parts of the world. The incidence of breast carcinoma in the Netherlands is one of the highest in the world with 130.05 per 100,000 females and 0.92 per 100,000 males (Dutch Cancer Registry 2008). This high incidence in the Netherlands may partly be caused by improved detection due to the nation-wide breast cancer screening programme for women between the age of 50 till 75 years old, who are invited for a mammography every two years. Considerable improvements in the overall survival of breast cancer patients have been observed over the past 15-20 years due to the better integration of local-regional and adjuvant therapeutic modalities. This is probably also the reason that breast cancer is (after lung cancer) the second most common source of central nervous system (CNS) metastases.[1] More effective treatment of primary breast cancer and of systemic (non-CNS) metastases of breast cancer will result in a increased number of patients that survive long enough to present with CNS metastases. Nowadays, all stages considered, approximately 75% of all breast cancer patients, can be expected to be alive ten years after diagnosis (Dutch Cancer Registry 2008).

In the past 5 decades a lot has changed in the treatment of breast carcinoma. Till the late 1960s, the treatment was focussed on the locoregional situation, i.e. the involved breast and ipsilateral axilla. The only way tumour growth could be controlled was by removing the whole breast (mastectomy) and axillary lymph node dissection, often with removal of underlying muscle tissue (musculus pectoralis major). Some of the patients subsequently received chest wall irradiation.

With the increasing number of treatment modalities for breast tumours it became increasingly important to establish the nature of the lesion prior to surgical excision/resection. This information was often provided by an excisional biopsy followed by an intra-operative frozen section diagnosis. Awakening from the anaesthesia, the patient discovered whether the breast was removed (cancer) or not (benign). Another diagnostic modality was the incision biopsy: a piece of the tumour was removed before surgical intervention. After preparation in the laboratory the tissue was microscopically examined (histological examination). Later, the fine needle aspiration (FNA) was introduced: with a small hollow needle loose cells or small clusters of cells were obtained by puncturing the tumour. After laboratory processing these cells were microscopically assessed (cytological examination).

The first report on the use of needle punctures is found in early Arab writings circa 1000 AD.[2] Martin and Ellis from Memorial Centre Hospital, New York, are considered to be the founders of modern needle aspiration techniques in the late 19th and early 20th century.[2-4] During the 1940s the interest in the thin and tick needle biopsy practice decreased after some reports on negative aspects of this biopsy practice.[4] The pioneers in the field of aspiration technique and diagnosis were never confident about the diagnosis of lymph node, thyroid and salivary lesions. This situation led to frequent misunderstandings amongst their clinical colleagues who therefore considered this technique as risky and unreliable. However, in the Memorial Centre Hospital, they continued to use this technique, but not for thyroid and

salivary glands lesions.[4] Later on, several clinicians and pathologists revived the needle biopsy technique as a tool for obtaining diagnostic material, amongst them the Dutch hematologist Dr. Paul Lopez Cardozo who published an atlas on clinical cytology in 1954.[5] A group at the Karolinska Hospital in Sweden, with especially Drs. Soderstrom and Zajicek, significantly contributed to the worldwide introduction of FNA as a diagnostic tool after the 1960s.[3]

Several improvements were made allowing better cytological analyses. Particularly during the last three decades, the improvements of cytological examinations of breast lesions consisted of a) better equipment for obtaining cell material for cytological analysis; b) improved processing of the FNA material; c) improved staining methods of the cell material.

Ad a: The cells are aspirated by means of a hollow needle connected with a syringe. The diameter of the needle varies and can for instance be 0.57mm (23 gauge) or 0.72 mm (21 gauge). With the introduction of the syringe holder the tumour could be more easily sampled in different directions in order to retrieve more representative cell material.[6] Furthermore, the use of ultrasound guided FNA increased the amount of diagnostic cells from palpable breast lesions and enabled aspiration of non-palpable lesions.

Ad b: traditionally, the cell material, obtained with a 21-or 23 gauge needle is expelled onto several glass slides by the aspirator. Next the material is gently smeared with another slide in order to get a good morphology without introducing crush artefacts. The slides can be air-dried and stained with May-Grunwald Giemsa (MGG) or the slides can be fixed with alcohol followed by the Papanicolaou (Pap) stain. This manner of processing is called the conventional smear preparation (CS).

The monolayer preparation (MP) technique, also called liquid-based cytology, was introduced initially as an alternative for cervical smears in the 1990s.[7, 8] However, many laboratories now also process other specimens, including FNA cytology, using this technology.[9] Automated devices, such as the ThinPrep processor, are readily available, but manual liquid-based techniques are less expensive and provide similar quality.[10, 11] Our pathology department introduced the manual MP technique, employing the Hettich cytocentrifuge (synonym: cytospin procedure). In this procedure, the aspirated cells are fixed in alcohol and centrifuged in a Hettich Rotina 48S® or Hettich Rotanta 46S® centrifuge *(Andreas Hettich GmbH & Co.KG, Tüttlingen, Germany).*[12] After discarding the supernatant the mixture is dripped into a bucket and clasped on a slide, creating a monolayer arrangement of cells within a 12 mm diameter area. The MP slides are Papanicolaou-stained. The procedure is described in more detail in chapter 2. The advantages of the MP technique approach are uniform aspirate processing with enhanced morphology, an increased number of all cells for microscopic evaluation, and the cells are deposited in a limited area on a single slide which means reduction of the screening time.[13]

Ad c: the conventional May-Grunwald Giemsa (MGG) and Papanicolaou stainings were improved by relatively subtle changes in the composition of these stainings. The

morphological analysis of the cytological specimen is now often supplemented by immunocytochemical staining. Additionally molecular analysis is increasingly used in diagnostic pathology to improve the detection of malignancy in FNAs.[14] The value of immunocytochemistry and molecular analysis are beyond the scope of this thesis.

The consensus recommendations for breast FNA as developed and approved by the National Cancer Institute (NCI)-sponsored conference approach in 1996 define the FNA as a reliable and efficient diagnostic tool for breast lesions when performed according to the protocol by experienced different disciplines (pathology, surgery, radiology).[15] These recommendations were also formulated in order to asses the position of the breast FNA facing the increasing use of core needle biopsies (CNB), generally using a 14 gauge needle (i.e.1.6 mm), in breast lesions as a first-line diagnostic modality in the diagnostic work-up of palpable and non-palpable breast lesions. A major advance of the CNB is that the resulting histology is generally more easy to interpret than cytological specimen and therefore can be interpreted by virtually any board-certified pathologist.[16]
In the NCI protocol, the FNA diagnoses are classified into one of 5 diagnostic categories: malignant (C5): cellular findings are diagnostic of malignancy, if possible it should be further characterized with the specific type of neoplasm; suspicious for malignancy (C4): the cellular findings are highly suggestive of malignancy; atypical/indeterminate (C3): the cellular findings may be atypical but are not diagnostic; benign (C2): no evidence of malignancy; inadequate (C1): due to scant material, artefact, obscuring blood or inflammation or otherwise.[15]

The studies presented in this thesis focus on the contribution of cytological examination in the diagnostic work-up and management of patients with breast lesions, especially for a 'same-day breast clinic', and on the value of cytological examination of the cerebrospinal fluid in order to detect central nervous system metastases in patients with breast carcinoma presenting with neurological symptoms.

Outline of thesis

In 1997 the monolayer preparation technique (MP) was introduced for FNA of breast lesions in our department. In **chapter 2** the conventional smear (CS) method was compared with the MP technique of breast FNA, employing the Hettich centrifuge.[10, 12] The FNA diagnoses were classified into one of the 5 diagnostic categories as proposed by the 1996 National Cancer Institute (NCI)-sponsored conference approach.[15] The reference standard was the histological follow-up. A conclusive FNA diagnosis was defined 'benign' on FNA as well as in the histological follow-up, or 'malignant' on FNA as well as in the histological follow-up. In this retrospective study the results of aspirates processed by CS and by MP were compared in different cohorts of patients.

According to the literature in 7 to 32% of cases an inconclusive FNA diagnosis (C1, C3 and C4) on breast lesions is given.[17-19] In those cases a repeat FNA or a core needle biopsy (CNB) will generally be performed in order to get a more definitive preoperative diagnosis. The objective of the study in **chapter 3** was to compare the results of repeat FNA and CNB with regard to their ability to provide a clinically more useful diagnosis after a first inconclusive breast FNA result.

Nipple discharge (ND) is the third most frequent complaint of patients visiting a breast clinic.[20-22] To the public ND is some sort of alarm announcing breast cancer.[22, 23] In many centres smears of ND are performed for it is easily to obtain and it may reveal a carcinoma of the breast. In **chapter 4** a retrospective study was performed to elucidate the diagnostic significance of the colour and the cytological examination of ND with a minimal follow-up period of 2 years. Some authors reported higher malignancy rates in bloody ND, whereas others found no association.[22-26] We investigated the correlation of the colour (esp. bloody vs. white) of the ND with the histological diagnosis and whether the latter is more accurate than the cytological examination of ND.

In the Netherlands the incidence of breast carcinoma in males is 0.92 per 100,000 (Dutch Cancer Registry 2008). In **chapter 5** the value of the FNA in the work-up of male breast lesions was determined in comparison with histological analysis over a period of 15 years. The results were compared with studies in the recent literature.[27-29]

Many laboratories replaced FNA by CNB. The histological diagnosis based on CNB gained importance in the initial diagnostic work-up for it can provide more information about the tumour characteristics such as precise type of carcinoma, hormonal receptor status and other molecular features.[30] Subsequently, some laboratories introduced core wash (CW) or touch imprint (TI) cytology from the CNB, aiming at a quick preliminary diagnosis that contributes to efficient management of the 'same-day breast clinic' and for alleviating patient anxiety.[31-35] Contradictory results of both techniques in the literature led to our preclinical study investigating an alternative modified method of TI and CW cytology (**chapter 6**).[31-33, 36-39] Fresh breast specimens (mastectomy, lumpectomy), obtained after surgery were biopsied by a core needle in a laboratory setting. With the modified technique TI and CW slides were made,

and categorized as malignant (C5), suspicious for malignancy (C4), atypical (C3), benign (C2) and inadequate (C1), and the results were compared with the histologic CNB results.[15]

The first results obtained in the clinic by CW cytological diagnosis in breast cancer patients are presented in **chapter 7**. The results of the CW cytology and CNB histology were correlated with the histopathology of subsequently obtained resection specimens and sensitivity and specificity of CW cytology was calculated.

Breast cancer is (after lung cancer) the second most common source of central nervous system (CNS) metastases. The incidence given varies between 3% to 15%.[1, 40-44] More effective treatment of primary breast cancer and of systemic (non-CNS) metastases of breast cancer will result in an increase in number of patients that survive long enough to present with CNS metastases.[1, 44-48] The neurological signs and symptoms of CNS metastases are widely variable and generally caused by increased intracranial pressure or neurological dysfunction due to local effect of the tumour. The neuroradiological findings are very divergent as well. Proof of metastatic disease in/around the CNS is important for rational therapeutic decision making (e.g. systemic and/or intrathecal chemotherapy, radiotherapy, wait and see policy). Cytology of cerebrospinal fluid (CSF) obtained by a lumbar puncture is generally considered as a quick, relatively easy, minimally invasive and inexpensive method to prove the presence or absence of metastatic spread to the CNS. In **chapter 8** the value of a CSF cytological diagnosis in breast cancer patients presenting with neurological signs and/or symptoms suspected for metastatic spread to the CNS were assessed. The results of cytological analysis of CSF were in this retrospective study compared with the clinical, neuroradiological, and survival data of these patients.

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Chapter 2

The role of laboratory processing in determining diagnostic conclusiveness of breast fine needle aspirations: conventional smearing versus a monolayer preparation

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J Clin Pathol 2009 62: 931-934

ABSTRACT

Background

To compare breast fine needle aspiration (FNA) specimens prepared by conventional smearing (CS) versus monolayer preparation (MP), with respect to the conclusiveness of the cytopathological diagnosis.

Methods

From 1992 to 1996, aspirators prepared aspirates themselves by direct smearing onto 2–4 slides. From 1999 to 2003, aspirate preparation was performed in the laboratory, creating a MP, using a Hettich cytocentrifuge. FNA diagnoses were categorised into inadequate (C1), benign (C2), atypical (C3), suspicious for malignancy (C4) and malignant (C5). The reference standard constituted histological follow-up. A conclusive FNA diagnosis was defined as C2 in lesions benign on follow-up and C5 in lesions malignant on histology.

Results

From 1992 to 1996, 692 aspirates were processed by CS, whereas from 1999 to 2003, 1301 aspirates were processed by MP. More FNA were ultrasound-guided in the MP group (85.6% versus 21.5%, $p<0.001$). When compared with CS, MP-prepared FNA had conclusive diagnoses significantly more often (72.8% versus 58.5%, $p<0.001$). This effect remained significant when corrected for the difference in ultrasound guidance (adjusted odds ratio 1.7, 95% confidence interval 1.3 to 2.2, $p<0.001$), and was larger for malignant lesions than for benign lesions (51.7% versus 79.9%, $p<0.001$).

Conclusion

Patients presenting with breast lesions can more often be offered a same-day, conclusive cytopathological diagnosis when FNA are prepared by a manual MP processing technique.

INTRODUCTION

The primary aim in same-day breast cancer diagnosis is integration of imaging, clinical investigation and cytological confirmation within 1 day. Consequently, it is important to minimise the number of inconclusive fine-needle aspiration (FNA) results. Liquid-based preparation of aspirates has been gaining popularity since its launch in the early 1990s. It has proven cost effective and time saving when compared with conventional smear (CS) preparation in gynaecological[1,2] and non-gynaecological[3] cytology. More important, it offers uniform aspirate processing, possibly decreasing the number of inadequate FNA, and thus precluding expansion of the diagnostic investigation.[4]

Technically, a liquid-based preparation differs from a CS in that the aspirate is immediately fixed and centrifuged and that cells end up on a limited area on a single slide. This implies a shorter screening time,[5] and also a change in cytological interpretation. Compared with CS, liquid-based preparations increase cellularity[6] and enhance cellular morphology.[5]

While automated devices, such as the ThinPrep processor, are readily available, manual liquid- based techniques are less costly and might there- fore prove a sensible alternative.[7]

In this paper, we report on our diagnostic experience with a manual monolayer preparation (MP) of breast FNA, employing the Hettich cytocentrifuge.

The present study aimed to evaluate the rates of conclusive diagnoses established by FNAs prepared by MP as compared with CS. Using retrospective data on 1993 cases, we hypothesised that breast FNA would be diagnosed conclusively more frequently when the aspirate was prepared by MP.

METHODS

Data collection

This retrospective study was performed on consecutive FNAs taken from palpable and non-palpable breast lesions in a regional teaching hospital, followed by a histological diagnosis. Data were extracted from the prospective national pathology database of The Netherlands.

Cytology specimens were obtained by radiologists using ultrasound guidance, and by surgeons using freehand aspiration. Either a 21 gauge or a 23 gauge needle was used for aspiration, and this was random for both CS-prepared and MP- prepared aspirates. No retrospective data on the aspirator's skill were available. Following excisional biopsy, tumour size was measured by pathologists, and the histological grade of malignant lesions according to the modified Bloom–Richardson classification[8] was determined.

Laboratory processing

CS and MP samples were processed in the same laboratory. CS preparation was routinely used from 1992 to 1996. From then, it was gradually replaced by MP, giving cytotechnologists and pathologists time to gain experience. From mid- 1998, MP became the only method used. Therefore FNAs taken in 1997 and 1998 were excluded from this study, thereby skipping the learning curve.

Conventionally prepared specimens included direct smears for solid masses and cytospins (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for fluids. For CS, the aspirate was smeared by the aspirator onto as many slides as were needed to allow a complete image of the specimen (ie, 2–4 slides) and instantly fixated in 95% ethanol. This allowed for immediate cytological examination when the slides arrived in the laboratory.
As for MP, the aspirator had no role in the cytological preparation. Once arrived in the laboratory, cells were rinsed from the needle and syringe into a vial and directly fixed in a 50% ethanol, 2% polyethylene glycol solution. The mixture was centrifuged for 10 min at 688 rpm in a Hettich Rotina 48S or Hettich Rotanta 46S cytocentrifuge (Andreas Hettich, Tuttlingen, Germany).[9,10] The supernatant was discarded. Depending on the estimated density of the sediment, one or two drops of the 50% ethanol, 2% polyethylene glycol solution was added. The resultant mixture was dripped into a bucket that was clasped on a poly-L-lysine slide (Menzel, Braunschweig, Germany). By centrifuging for 5 min at 688 rpm, the sediment was pressed onto the slide, creating a monolayer arrangement of all cells within a 12 mm diameter area. Only one MP slide per aspirate was prepared.[4] In case of a haemorrhagic aspirate, an additional slide was prepared by lysing erythrocytes with 2.5% acetic acid. CS and MP slides were stained with Papanicolaou stain.

FNA examination and histology

Slides were screened by cytotechnologists and examined by 10 staff cytopathologists with 6–20 years of experience in cytopathology. Using simple and well-defined criteria,[11] they classified each aspirate into one of five diagnostic categories: inadequate (C1), benign (C2), atypical (C3), suspicious for malignancy (C4) and malignant (C5).[12] A conclusive FNA diagnosis was defined as a benign diagnosis (C2) in lesions that were benign on follow-up and as a malignant diagnosis (C5) in lesions that were malignant on follow-up. On occasion, clinical information regarding the breast lesion was available to the pathologists at the time of cytopathological examination.
Each aspirate was related to its eventual histological diagnosis. Invasive carcinoma and ductal carcinoma in situ (DCIS) were considered malignant. All other diagnoses were considered benign.

Statistics

The relationship between the method of preparation (CS versus MP) and diagnostic conclusiveness was described with an odds ratio (OR) and was corrected for the effect of possible confounders by logistic regression. We used the $x2$ test for testing differences between proportions of FNA diagnostic categories between the CS and MP group.
We a priori planned one subgroup analysis. Since the elimination of disturbing background material is an important feature of MP,[13] we hypothesised that the effect of MP on diagnostic conclusiveness would be larger in malignant lesions. We tested this with a formal test of interaction, using logistic regression.[14]
All tests were two-sided. Values of $p<0.05$ were considered significant. When separately testing proportions of FNA diagnostic categories, we adjusted the significance threshold to $p<0.01$ for multiple testing.[15]

RESULTS

Study population

From 1 January 1992 to 31 December 1996, 692 FNA were performed and subsequently prepared by CS. From 1 January 1999 to 31 December 2003, 1301 FNA were performed and prepared by MP. Of this subsequent total of 1993 lesions aspirated, 516 (25.9%) were benign and 1477 (74.1%) were malignant. The most frequent histological diagnoses included invasive ductal carcinoma (n = 1073), aspecific benign tissue (n = 241), invasive lobular carcinoma (n = 223) and fibroadenoma (n = 141).

Table 1 shows comparative clinical and pathological characteristics of the studied subjects. The only marked difference between the groups was that breast lesions in the CS group were significantly less likely to be aspirated under ultrasound guidance than those in the MP group.

Table 1 Key patient characteristics

	Preparation	
Characteristic	CS (n = 692)	MP (n = 1301)
Mean age (SD), years	55.9 (14.8)	55.9 (15.0)
Malignant, %	72.7	74.9*
Mean tumour size (SD), mm	24 (17)	22 (13)*
Tumour grade, %†		
Grade 1	16.2	14.8
Grade 2	44.6	44.9
Grade 3	38.8	40.0
Unknown	0.4	0.3
Ultrasound-guided FNA, %	21.5	85.6‡

*p<0.05; †in malignant tumours only; ‡p<0.001.
CS, conventional smear; FNA, fine needle aspiration, MP, monolayer preparation

Diagnostic conclusiveness

MP-prepared aspirates were conclusive significantly more frequently than aspirates prepared by CS (72.8% versus 58.5%, respectively, OR 1.9, 95% confidence interval (CI) 1.6 to 2.3, p<0.001; Table 2). When corrected for the effect of ultrasound guidance, a slightly weaker, but still highly significant, association remained (adjusted OR 1.7, 95% CI, 1.3 to 2.2, p<0.001). This corrected beneficial effect of MP on conclusiveness was significantly more pronounced in malignant lesions than in benign lesions (p<0.001, Table 3).

Specifically, MP-prepared aspirates of benign lesions were significantly less often inadequate than were CS-prepared aspirates (18.0% versus 32.8%, respectively, p<0.001; Fig. 1).

Table 2 Association between the method of preparation and diagnostic conclusiveness

Preparation	Conclusive*	Inconclusive
Controventional smear	58.5	41.5
Monolayer preparation	72.8	27.2†

Values are percentages.
*Defined as a C2 diagnosis in benign lesions and a C5 diagnosis in malignant lesions.
†1.9, 95% CI 1.6-2.3, p<0.001.

Malignant lesions had significantly less inadequate (4.8% versus 17.7%, respectively, p<0.001), less suspicious (7.2% versus 11.7%, respectively, p = 0.005), and more malignant FNA diagnoses (79.9% versus 63.4%, respectively, p<0.001) when the aspirate was prepared by MP

Table 3 Subgroup analysis of the effect of the preparation method on the diagnostic conclusiveness of fine needle aspiration in malignant and benign lesions

	Histologic (conclusiveness)	
Preparation	Benign	Malignant
Conventional smear	45.5	63.4
Monolayer preparation	51,7	79.9*

Values are percentages.
*p<0.001 for the effect of the interaction between preparation method and histology on diagnostic conclusiveness

DISCUSSION

Key findings and limitations

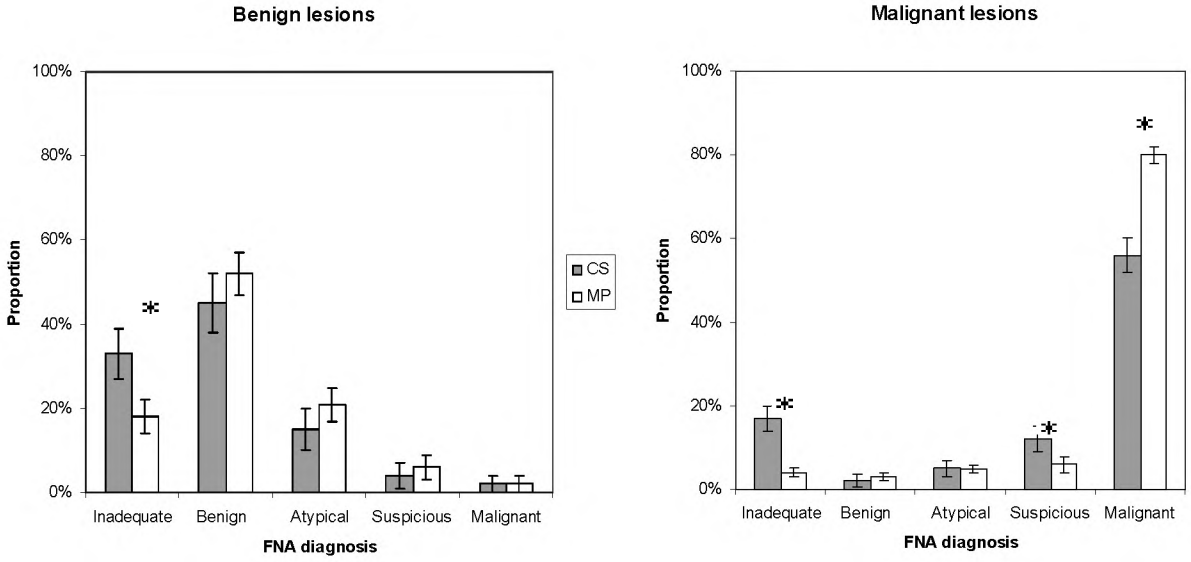
The current study demonstrates that (1) breast FNAs are more conclusively diagnosed when processed by MP compared with CS, and (2) this benefit is most pronounced in malignant breast lesions. Our data show a 14.3% absolute reduction of inconclusive FNAs following MP adoption. This indicates that for one additional FNA to be diagnosed conclusively, seven aspirates should be processed by MP instead of CS (1/0.143).

The major limitation of this study lies in its retrospective nature. Certain confounding factors, such as differences among aspirators and variable cytopathologist experience, cannot be controlled for. Hence, a direct and completely fair comparison between CS and MP is impossible. However, we accounted for key patient and tumour characteristics affecting conclusiveness; these characteristics included ultrasound guidance, tumour size and tumour grade.[16,17]

Inconclusive cytological diagnoses

Inconclusive FNA diagnoses (ie, diagnoses other than 'benign (C2)' in benign lesions, and other than 'malignant (C5)' in malignant lesions) are of clinical concern. These provide no information, delay diagnostic investigation, and require further investigations, such as core needle biopsies, to provide a conclusive diagnosis.[18–20] Low-grade tumours and increased medicolegal claims are acknowledged as causes of the increased reporting of indeterminate diagnoses.[11] Several studies have demonstrated variable conclusiveness rates for CS, ranging from 54% to 87%.[21–24] As for MP, conclusiveness rates of 73–77% have been reported for breast FNAs processed by MP.[7,25] Our results correspond well with these findings (Table 2). Liquid-based preparation techniques, such as ThinPrep, have received considerable attention because of their favourable results with respect to cytomorphology and diagnostic conclusiveness.[5,13,24] In direct comparisons with CS, ThinPrep has been shown to yield superior conclusiveness in gynaecological,[3,26] general non- gynaecological[3] and breast lesions.[24] However, no direct comparisons of CS and MP preparations with regard to validity have been performed. In a paired comparison of 44 breast lesions, Florentine et al found CS and MP to yield similar cytological diagnoses, correlating in 41 cases.25 However, FNA results were not correlated with histology.

Figure 1 Frequencies of fine needle aspiration (FNA) diagnoses of aspirates prepared by conventional smearing (CS) and by a monolayer preparation (MP), in benign and malignant breast lesions. Data are presented as means and 95% confidence intervals. *p<0.01 for the difference in the proportions of a diagnosis between CS and MP.



Obviously, the laboratory preparation method is not the only determinant of whether an aspirate will yield a conclusive, valid diagnosis. Substantially more aspirations were ultrasound- guided in the MP group. Therefore, lesions in this group may have been aspirated more accurately.[16,27] Yet, conclusiveness of MP-processed aspirates remained significantly

higher following statistical correction for the effect of ultrasound guidance. This implies a genuine effect of MP on conclusiveness. Moreover, the finding of a smaller mean tumour size in the MP group, possibly caused by the implementation of the Dutch nationwide breast cancer screening programme,[28] may partly counterbalance the relative beneficial effect of ultrasound on conclusiveness. Detection of smaller tumours may have contributed to increased difficulty in obtaining diagnostic aspirations in the MP group.[17]

Differences in preparation and cytomorphology

During MP processing, aspirated cells are centrifuged and are concentrated upon a single 12 mm slide. This results in a higher observed cellularity, whereas in CS cells are dispersed over multiple slides.[25,29] Additionally, liquid-based preparation comprises direct rinsing of cells in fixation fluid, preventing air drying artefacts and removing disturbing background material, both of which are frequently present in CS-prepared aspirates.[30] Finally, conventionally smeared cells may be destroyed in the smearing process. These CS drawbacks are probably crucial in cases of borderline adequacy. For example, liquid-based preparations, including MP, remove the majority of disturbing background material such as necrotic debris.[6,13,29] As the latter is relatively frequently present in aspirates of malignant lesions,[31] the effect of MP may be more pronounced here. Indeed, we found that FNA conclusiveness increased more substantially in malignant lesions than in benign lesions following the implementation of MP.

Despite the potential advantages as described above, MP has potential limitations. First, MP of breast aspirates requires laboratory processing and therefore more time than CS. Yet, this difference is in the order of 20 min and should therefore not compromise the ability to provide a same-day diagnosis by FNA. A second potential limitation of MP is that is does not allow for an on-site assessment of aspirate adequacy by methods such as Diff-Quik.[32] In contrast, this is possible for smears. Thus, FNA in the CS group may have been conclusive more often had every aspirate been pre-assessed for adequacy. However, routinely performing these assessments is costly and time consuming,[33] resulting in few centres adhering to this protocol.

Conclusion

In conclusion, patients presenting with palpable and non- palpable breast lesions can more often be offered a same-day diagnosis when FNA are prepared by a manual MP processing technique. Consequently, breast clinics adhering to conventionally smeared FNA as a means of providing a same-day, preliminary diagnosis of breast lesions may benefit from switching to MP processing.

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Chapter 3

Indeterminate Breast Fine-Needle Aspiration: Repeat Aspiration or Core Needle Biopsy?

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ABSTRACT

Background

Fine-needle aspiration (FNA) of breast lesions provides indeterminate (C1, C3, and C4) diagnoses in a high proportion of cases. The aim of the present study was to retrospectively determine whether repeat FNA or core needle biopsy (CNB) most frequently provides a correct and more conclusive diagnosis.

Methods

All patients who had an indeterminate primary FNA followed by repeat FNA or CNB within 1 month from 1992 to 2007 were included. FNA was diagnosed as C1–C5; CNB was diagnosed as B1– B5. Improvement in preoperative diagnosis by repeat FNA or CNB was defined as C2/B2 in benign lesions, C3/B3 in premalignant lesions, C4/B4 or C5/B5 in malignant lesions where primary FNA was C1, and C5/B5 in malignant lesions where primary FNA was C3 or C4.

Results

Among 255 eligible cases, CNB improved the preoperative diagnosis more often than did repeat FNA (78.0% vs. 54.8%, odds ratio = 2.9, P<.001). When corrected for patient age, appearance on mammogram (mass or not), clinical findings (palpable or not), tumor size, and aspiration mode (freehand vs. image guided), this difference slightly increased (odds ratio = 3.0, P = .001).

Conclusion

CNB should be performed after an indeterminate FNA of a breast lesion to obtain a reliable and clear preoperative diagnosis.

INTRODUCTION

Fine-needle aspiration (FNA) has proven to be a reliable asset in same-day diagnosis of breast malignancies.[1–3] Given the relative increase in nonpalpable breast lesions as a result of nationwide breast cancer screening, FNA carries more consequences in treatment planning than it did formerly. However, no definitive diagnosis can be established on the basis of cytology in 7% to 32% of cases.[4–6] This group comprises lesions with inadequate (C1), atypical (C3), and suspicious (C4) FNA. Routinely, either a repeat FNA or a core needle biopsy (CNB) is performed to obtain a more definite preoperative diagnosis.[5–7]

The objective of the current study was to compare repeat FNA and CNB with regard to their ability to provide a clinically more useful diagnosis after an indeterminate primary breast FNA.

The hypothesis was that when corrected for potential confounding variables, CNB allows for a true and conclusive preoperative diagnosis more often than repeat FNA. This was tested by multivariate logistic regression analysis of 255 cases.

MATERIALS AND METHODS

Study Population and Data Acquisition

Data on all patients visiting the one-stop breast clinic in a regional teaching setting between 1992 and 2007 were retrospectively collected from the Dutch national pathology database PALGA. Patients that had an indeterminate FNA diagnosis (C1, C3, or C4) followed by a repeat FNA or a CNB of the same breast lesion within 1 month were analyzed.[8] The PALGA database and patient files were reviewed for data on patient age, aspiration mode (freehand vs. image guided for both FNA and CNB), tumour size, clinical findings (palpable or nonpalpable), type of dominant lesion on mammography (mass or not), and signs of ipsilateral breast cancer developing if the patient was not operated on. Nonmass mammographic lesions included microcalcifications and architectural distortions. Tumour size was measured on the surgical specimen or on ultrasound in cases where the patient was not operated on.[9]

Follow-Up

The reference standard constituted surgical specimens or clinical follow-up when no surgical outcome was available. Premalignant surgical lesions included atypical ductal hyperplasia, lobular carcinoma-in-situ, and atypical papillomatosis.

Repeat FNA and CNB

Cytologic (FNA) and histologic (CNB) material was obtained by freehand or image-guided needle handling. For FNA, a 21- to 23-gauge needle attached to a 20-mL syringe was used. FNA were diagnosed as C1, inadequate; C2, benign; C3, atypical; C4, suspicious; or C5, malignant.[8,10] An indeterminate FNA diagnosis was defined as C1, C3, or C4. Aspirates were either smeared and fixed in ethanol 95% or rinsed, fixed in an ethanol 50%–polyethylene

glycol 2% solution, and centrifuged, creating a monolayer preparation. Both smears and monolayer slides were Papanicolaou stained.

CNB were performed with an 18-gauge needle mounted on an automated device (Bard Magnum, C. R. Bard, Covington, GA) for lesions appearing as masses on ultrasound. For lesions invisible on ultrasound, stereotactic vacuum-assisted CNB (Vacora, Bard Peripheral Vascular, Tempe, AZ) were performed with a 14-gauge needle. CNB specimens were fixed in formalin and stained with hematoxylin and eosin. CNB diagnostic categories included B1, normal tissue or unsatisfactory; B2, benign; B3, uncertain malignant potential; B4 suspicious of malignancy; and B5, malignant.[8] B3 lesions included atypical ductal hyperplasia, lobular carcinoma-in-situ, and atypical papillomatosis. FNA and CNB were examined by 12 pathologists with 11 to 20 years of experience at the beginning of the study period. Limited clinical information was available to pathologists when examining the slides.

A preoperative improvement of an indeterminate FNA diagnosis was noted when repeat FNA or CNB yielded a true and more conclusive diagnosis. In exceptional cases, lesions diagnosed as premalignant on the surgical specimen with inadequate and suspicious diagnoses on primary FNA and atypical diagnoses on repeat FNA or CNB were considered to have had an improvement in preoperative diagnosis (Table 1).

Table 1 Criteria for assigning improvement in preoperative diagnosis according to histologic nature of surgical specimen

Follow-up	Indeterminate diagnosis	Improved preoperative diagnosis	
	First FNA	Second FNA	CNB
Benign	C1, C3, C4	C2	B2
Premalignant	C1, C4	C3	B3[a]
Malignant	C1	C4, C5	B4, B5
	C3, C4	C5	B5

FNA fine-needle aspiration, CNB core needle biopsy

[a] Includes atypical ductal hyperplasia, lobular carcinoma-in-situ, and atypical papillomatosis

Statistical Analysis

The x^2 test was used for comparing proportions of diagnostic improvement between repeat FNA and CNB. A forced-entry logistic regression model was used for correcting this difference for patient age, tumour size, aspiration mode, and lesion type as assessed by mammogram, as well as whether or not the lesion was palpable. Differences and odds ratios (OR) were considered to be statistically significant at $P<.05$. SPSS version 15.0 (SPSS, Chicago, IL) was used for statistical analysis.

RESULTS

Patient Characteristics

From January 1, 1992, until December 14, 2007, a total of 255 FNA in 242 patients met the inclusion criteria. No patient received neoadjuvant therapy. Mean patient age was 55.6 years (standard deviation, 13.0). Follow-up was derived from the surgical specimen in 198 cases (median time to surgery, 5 days; range, 0–122 days) and from clinical follow-up in 57 cases (median duration, 43 months; range, 7–137 months). Of these latter patients, 12 had malignant disease, but they were not operated on because they had distant metastases (n = 3), refused (n = 2), or were deemed unfit for surgery (n = 5), or because the aspirated lesion was a metastasis from a nonmammary primary tumor (n = 2).

After the primary indeterminate FNA, 73 patients had repeat FNA and 182 patients had CNB. At follow-up, 87 lesions turned out to be benign, 10 were premalignant, and 158 were malignant.

Table 2 Univariate differences in allowance for diagnostic improvement by repeat FNA vs. CNB after an indeterminate primary FNA.

Second-line diagnostic modality	Diagnostic improvement (%)	
	No	Yes
FNA	45.2	54.8
CNB	22.0	78.0[a]

FNA fine-needle aspiration; CNB core needle biopsy

[a] $x^2 = 13.6$, $P < .001$

Diagnostic Improvement

CNB improved the preoperative diagnosis more often than did repeat FNA (78.0% vs. 54.8%, OR = 2.9, $x^2 = 13.6$, $P < .001$, Table 2). When adjusted for patient age, tumour size, and appearance on mammogram, as well as whether or not the lesion was palpable and the aspiration mode of the first and second diagnostic procedures, CNB still performed better compared with repeat FNA (OR = 3.0, P = .001, Table 3). Logistic regression revealed no significant effect of any of the above parameters on improving preoperative diagnosis. There were no significant correlations between tumour size and clinical findings (P = .23) and between appearance on mammogram and clinical findings (P = .16).

DISCUSSION

The current study evaluated the optimal preoperative workup after an indeterminate (C1, C3, or C4) breast FNA. In patients who subsequently had CNB, preoperative diagnosis improved in 78.0%, compared with 54.8% for patients with repeat FNA (Table 2). This difference was not affected by clinical, radiologic, and histopathologic differences between these groups (Table 3).

Table 3 Multivariate logistic regression analysis of factors affecting diagnostic improvement

Factor	Odds ratio[a]	*P* value
Repeat FNA vs. CNB	3.0	0.001
Patient age	1.0	0.17
Palpable vs. nonpalpable	0.8	0.70
Mass vs. no mass on mammography	0.7	0.36
Tumor size	1.0	0.90
Primary FNA, freehand vs. image guided	1.3	0.54
Repeat FNA/CNB, freehand vs. image guided	1.6	0.38

FNA fine-needle aspiration; CNB core needle biopsy

[a] An odds ratio of >1 indicates an increased corrected chance of improving the preoperative diagnosis when the covariate increases with one unit (i.e., switches from the first to the second category in case of a dichotomous covariate)

In general, CNB is preferred over FNA as the first-line diagnostic modality because it more often provides a conclusive and adequate diagnosis than FNA.[4,11–13] However, other centers continue to use primary FNA because the predictive value of malignant FNA diagnoses approaches 100%, allowing for a same-day diagnosis in 49% to 90% of breast cancers.[1,2,6,14] Patients are served well when quickly informed about their diagnosis and treatment opportunities, but this is only possible when an unequivocal diagnosis is available preoperatively. Indeterminate FNA diagnoses result from sampling errors, interpretation errors, and possibly adverse histopathologic tumour characteristics such as a fibrous consistency and lobular histology.[15,16]

In these instances, either a repeat FNA or a CNB is routinely performed, probably varying with specific center protocols or radiologist preference.[6,7] However, the validity of this choice has only been defined in small patient groups. CNB has been reported to provide clinically useful information in 76% to 90%, compared with 41% to 45% for repeat FNA.[5,6,17] The present study indicates that this difference is somewhat smaller, but still evident. Compared with FNA, CNB needles are larger and sampling is less operator dependent.[18] Deep, desmoplastic lesions would therefore be unlikely to be aspirated successfully by repeating the same FNA procedure. Because most failed FNAs are the result of sampling error, this is probably the most important explanation of the observed difference in improvement of preoperative diagnosis.[6,19]

It is of note that CNB yielded clinically useful information in only 78%, while reported sensitivities and specificities are generally higher, ranging from 88% to 100% and 68% to 96%, respectively.[12,13,20,21] This is probably the result of a selection bias created by the study inclusion criteria, leaving only those lesions that are hard to aspirate or whose tissue is hard to examine by the pathologist. This might prompt some clinicians to directly move on to excisional biopsy after an indeterminate FNA. Although the current study did not investigate this scenario, it would not be advisable. A malignant CNB may result in wider margins at initial excision, possibly decreasing the chance of positive resection margins, and signs of histopathologic invasion on CNB may preliminarily determine axillary workup (i.e., sentinel node biopsy or not).[22,23] Furthermore, a negative CNB spares the patient unnecessary surgery, making it a cost-effective modality.[24]

However, indeterminate diagnoses remain after CNB. An atypical CNB diagnosis (B3) would still seem more valuable clinically than an atypical FNA diagnosis (C3) because B3 lesions on CNB carry highly variable risks of malignancy on subsequent excision.[25] As a consequence, treatment of indeterminate lesions can be more reliably tailored to the patient compared with situations where repeat FNA was atypical.

The present study has several limitations. Its retrospective design may not have allowed for a fair comparison between the repeat FNA and CNB groups. Because the study spans a long period of time, factors that have changed in time may have influenced diagnostic performance in these groups unequally. Multivariate analysis was used to minimize this potential bias. Yet the difference in diagnostic improvement between repeat FNA and CNB remained grossly unchanged between univariate and multivariate analyzes (OR = 2.9 to OR = 3.0, respectively; Tables 2, 3). This is rational to some extent. For instance, tumour size does not change from the first to the second diagnostic procedure and should therefore not affect better performance of one modality over the other.

Conclusion

In summary, this study indicates that a CNB should be performed after an indeterminate (C1, C3, or C4) FNA of a breast lesion to obtain a reliable and clear preoperative diagnosis. For breast clinics more heavily adhering to a same-day diagnosis, a repeat FNA may be feasible, but one should consider that obtaining a more conclusive diagnosis is only possible in half of these cases.

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Chapter 4

The diagnostic value of nipple discharge cytology in 618 consecutive patients

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ABSTRACT

Background

Preoperative stratification of patients presenting with nipple discharge (ND) according to malignancy risk has proven difficult. Nevertheless, cytological examination is considered to be a diagnostic aid. The aim of this study was to determine its complementary value in clinical decision-making in patients presenting with ND.

Methods

We retrospectively collected data on macroscopic ND colour, ND cytology, physical examination, mammography, ultrasound and fine-needle aspiration cytology results. On ND cytology, benign diagnoses were considered negative, whereas suspicious and malignant diagnoses were considered positive for malignancy.

Results

From 1992 to 2006, 618 patients had an ND smear, of those 163 patients had a biopsy. Sensitivity and specificity were 16.7% and 66.1%, respectively. These values were lower when ND was bloody than when ND was non-bloody ($p=0.66$ and $p<0.05$ for sensitivity and specificity, respectively). When macroscopically defining bloody ND as positive and non-bloody ND as negative, macroscopic ND colour examination had a remarkably higher sensitivity (60.6 vs. 18.2%, $p < 0.001$) and only a slightly lower specificity (53.6 vs. 65.0%, $p=0.07$) when compared to cytological ND examination. Only 1 malignant lesion was designated positive solely by ND cytology (unique sensitivity (95% CI), 2.8% (0.0-8.4%)) and 3 lesions were correctly classified as negative by ND cytology (unique specificity (95% CI), 1.6%, 0.0-3.7%)).

Conclusion

Nipple discharge cytology has little complementary diagnostic value. Therefore, its routine use for detection of ND-related breast pathology should be reconsidered carefully. Nipple discharge cytology may redirect patient management well in some cases, but it may confuse work-up in the majority.

INTRODUCTION

Nipple discharge (ND) is the third most frequent complaint of patients visiting a breast clinic, being the presenting symptom in 4-7% of cases.[1-3] Additionally, it accounts for 6-7% of breast surgical indications, ranking only second to a lump.[4,5] To the public the occurrence of ND has been ringing an alarm bell announcing breast cancer. However, preoperative risk stratification by a minimally invasive diagnostic work-up has proven difficult.[3,6] In the past decades the concept of pathologic ND has been introduced for this purpose.

Yet, controversy remains concerning the criteria of pathologic ND. Several studies regard unilateral, single-duct, spontaneous and persistent discharge as pathologic [6-11] As others include expressible[1,5,12,13] and bilateral ND [4,14] as well, reported malignancy rates in pathological discharges range from 6-29%.

In addition, it has been questioned whether discharge colour correlates to histological diagnosis. Some authors reported higher malignancy rates in bloody ND,[6,13] whereas others found no association.[8,11,14]

Traditionally, pathologic ND is evaluated by cytological examination of fluid smears. Although there are additional diagnostic clues in a majority of cases,[4,15] ND smears may detect malignancy reliably when other clues are absent. It is questionable however, how often this is the case.

The unique contribution of cytology to diagnosing pathologic ND has never been investigated. The present study aimed to retrospectively evaluate this by correlating cytological and histological diagnoses in cases of unequivocal positive or negative signs found by other diagnostic modalities. A further hypothesis was that solely examining macroscopic ND colour would at least be as accurate as the cytological examination of an ND smear.

MATERIALS AND METHODS

Study population

We included all patients presenting with unilateral spontaneous and expressible ND from whom material for cytology was obtained. As spontaneous ND may be temporarily absent at clinical presentation, we also included expressible ND. The patients were seen in a regional teaching and a regional non-teaching hospital. Following exclusion of patients that were pregnant (n=9), lactating (n=3) or had bilateral discharge (n=98), a 618-patient series remained eligible for analysis. We did not include patients with bilateral ND since this does not usually raise clinical suspicion of focal breast pathology. We included only patients who had ND smears until August 1, 2006, allowing for a minimal follow-up of 2 years. We chose this time interval because more than 40% of ND-secreting breast cancers in our group was excised more than 1 year following the ND smear.

Data collection

We searched patient files for clinical and radiological findings. We extracted pathology data from the Dutch nationwide pathology data network and archive (PALGA).

We noted the macroscopic colour of the discharge. For purposes of comparing diagnostic accuracies, we considered bloody ND as suspect for malignancy. A positive physical examination was defined as either a palpable mass, nipple retraction or pagetoid nipple aspect. Mammographic criteria for malignancy included irregular microcalcifications or a spiculated or irregular mass, whereas well-demarcated masses, polycystic architectural disturbance and gross microcalcifications were considered benign lesions. Ultrasound malignancy criteria were echogenic lesion within a dilated duct or a mottled hypoechoic architectural disturbance. We considered duct dilatation without a mass as benign.

ND acquisition and interpretation

Surgeons obtained ND by gently pressing the areola. ND was directly smeared onto a specimen slide and either fixated in ethanol 95% and Papanicolaou-stained, or air-dried and Giemsa-stained.

Pathologists assigned each smear one of the National Cancer Institute-recommended diagnostic categories: benign, atypical, suspicious or malignant.[16] A specimen was diagnosed 'benign' if no or few epithelial cells were present. Specimens diagnosed 'malignant' contained several dyscohesive clusters of anaplastic cells and, optionally, a necrotic background. For accuracy calculation, benign ND was considered negative for malignancy, while both suspicious and malignant ND were considered positive. Although not conclusive, suspicious cytology leads to further diagnostic evaluation, in fact bearing the same consequence as a malignant diagnosis.

Histology

We correlated smears to their later histological diagnosis if established within 5 years following cytology. Ductal carcinoma in situ (DCIS) and invasive ductal and lobular carcinoma (IDC and ILC, respectively) were considered malignant. In spite of carrying increased risks of malignant degeneration,[17-19] we designated atypical ductal hyperplasia (ADH) and papillomas as benign.

Twelve staff pathologists with 6-20 years of cytopathological experience examined both cytological and histological specimens. Clinical information or a prior cytological diagnosis was available to the pathologists.

Statistical analysis

We calculated sensitivity and specificity of ND cytology, as well as unique sensitivity and specificity. We defined unique sensitivity as the proportion of malignancies that was detected solely by cytology and unique specificity as the proportion of benign breast lesions that was designated malignant by examination, mammography and ultrasound, but benign by cytology.

We ran Chi-square and Fisher's exact tests for comparing proportions, whereas 95% confidence intervals (95% CI) for proportions were calculated by the Cloppere Pearson method.[20] We used SPSS 14.0 software (SPSS Inc., Chicago, IL) for calculations.

RESULTS

Patients' characteristics and histology

From January 1, 1992 to August 1, 2006, 618 eligible ND smears were made. During this period approximately 24,000 new patients had visited the study centres.
In this group median patient age was 50 years (range, 20-86) and 614 patients were women. Of these, 163 had histological follow-up, which we further analysed. Histology comprised excision or mastectomy in 160 cases and core needle biopsy in 3 cases. Median time between cytology and histology was 50 days (range, 0-1627). Overall, solitary papilloma was the most frequent surgical finding (n=51). Malignancy was identified in 36 cases in this group, of which 19 tumours were DCIS, 2 were pT1a, 3 were pT1c, 7 were pT2, 3 were pT3 and 2 were pT4b. Five tumours were grade 1, 12 were grade 2 and 19 were grade 3. No previous diagnoses of breast cancer had been established in these women.

Overall diagnostic accuracy

Measures of cytological diagnostic accuracy are noted in Table 1. ND smears detected 5 of 36 malignancies and 84 of 127 benign lesions (Table 1). Malignant lesions showed somewhat higher rates of positive and atypical cytological diagnoses. Nevertheless, ND smear diagnosis did not significantly predict the histological nature of the ND-causing breast lesion (x^2 = 5.0, p= 0.082).

Table 1 Smear diagnoses in malignant and benign breast lesions presenting with nipple discharge (n = 163).

Cytological diagnosis	Histology	
	Benign	Malignant
Benign	66.1 %	50.0 %
Atypical	27.6 %	33.3 %
Positive	6.3 %	16.7 %

ND colour

In smears with available ND colour and histology data (n=156), cytological analysis of bloody ND had similar sensitivity (Fisher's exact p=0.66) and lower specificity (x^2 = 7.2, p= 0.007) when compared to otherwise coloured ND (Table 2).

Table 2 Smear accuracy in cases of bloody and non-bloody discharge (n = 156).[a]

	Bloody	Non-bloody
Sensitivity	15.0 %	23.1 %
Specificity	52.6 %	75.8 % [†]

[†] $p < 0.01$ for smear specificy in bloody vs. non-bloody discharge.
[a] For cases with available colour data only.

Table 3 shows comparative sensitivities and specificities of macroscopic examination of ND colour alone and ND cytology in the same group as was analyzed in Table 2. The sensitivity of colour examination was higher than the sensitivity of cytological examination ($x^2 = 12.4$, $p < 0.001$). For specificity, no significant difference was found ($x^2 = 3.3$, $p = 0.069$).

Table 3 Diagnostic accuracy of macroscopic discharge colour and cytology (n = 156).[a]

	ND colour[b]	Cytology
Sensitivity (95 % CI)	60.6 % (43.0-78.2 %)	18.2 % (4.3-32.1 %)[‡]
Specificity (95 % CI)	53.6 % (44.7-62.6 %)	65.0 % (56.5-73.6 %)

[‡] $p = 0.001$.
[a] For cases with available colour data only.
[b] When regarding red discharge as positive and non-red discharge as negative.

Complementary diagnostic value

In the histology group data on clinical examination were available for all 163 cases. Mammography, ultrasound and FNA were performed in 155, 106 and 10 cases, respectively. Sixty-one patients had a lump on clinical examination. No breast lesion was detected by physical examination and imaging in 50 cases. Of these patients, 8 (16%) turned out to have breast cancer. Of all malignant lesions, 8 were designated benign by each of these diagnostic modalities, if performed (Table 4). Of these, 1 ND smear was still able to detect malignancy, thus carrying a unique sensitivity (95% CI) of 2.8% (0.0-8.4%). In addition, 4 smears were diagnosed as atypical, apparently necessitating histological biopsy in these cases. In 2 benign lesions the ND smear was the only negative diagnostic finding, indicating a unique specificity (95% CI) of 1.6% (0.0-3.7%). Yet, no mammography was performed in 1 of these cases.

DISCUSSION

The present series shows that ND smears had a very low complementary diagnostic value in both benign and malignant breast lesions that cause pathologic ND. Still, smears of ND are performed in many centres because they may incidentally detect a carcinoma.[9,11,14,21-23] The rationale for this is that although cytology carries a low sensitivity, a positive finding is alarming all the same because of its acceptable specificity. Furthermore, the ease of obtaining ND and preparing a smear has led to its common use for cytological examination.

Potential advantages of ND cytology

The group where the potential advantage of cytology lies is small but interesting for it includes those patients with discharging lesions that lie too deep for detection by palpation or too well hidden in a dense breast for conventional imaging.[2,24,25] Early cytological detection of breast cancers in this group may limit the extent of surgical and adjuvant therapy and improve prognosis. Nevertheless, relatively low malignancy rates are found in these patients in the present (16%) and other studies (0-13%),[6,9,11,26,27] which partly explains the low unique sensitivity of cytology. Over a 14.5-year period, with an estimated total of 1450 breast

Tabel 4 Diagnoses of nipple discharge smears in cases with concordant findings on physical examination, mammography, ultrasound and fine-needle aspiration.

	Histology	
	Benign (n = 127)	Malignant (n = 36)
All other modalities[a] positive	2	2
Negative cytology	2	2
Atypical cytology	0	0
Positive cytology	0	0
All other modalities[a] negative	76	8
Negative cytology	48	25
Atypical cytology	25	3
Positive cytology	3	1

[a] Examination, mammography, ultrasound and fine-needle aspiration (if performed).

cancers being treated in the two study centres, only one patient with breast cancer received timely surgery solely due to positive cytology (2.8% of all breast malignancies presenting with ND). In other studies on ND cytology unique sensitivities of 5-7% were found.[12,14] Cytology yielded the only negative finding in 2 out of 127 benign lesions, but these patients were still operated on. This indicates ignorance of negative cytology findings if they are not concordant with other diagnostic findings, as has been advocated before.[23,28,29]

Other diagnostic modalities

Actually, addressing ND with cytology may prove to be a futile adjunct to simple and more accurate diagnostic aids. Of patients with unilateral ND there is no clinical and radiological evidence of a breast lesion in only 25%. Reported sensitivities of physical examination, mammography and ultrasound range from 5-94%, 10-91% and 36-83%, respectively, whereas specificities of 50-93%, 38-99% and 12-68% have been reported, respectively.[1,4,5,7,8,10,11,24,26,30] Although mammography and ultrasound are independent predictors of malignancy,[11] cytology is not (Table 1), as emphasized by its low unique sensitivity and specificity (Table 4).

When disregarding other diagnostic modalities, cytology has an extremely low sensitivity (16.7%) and a low specificity (66.1%, Table 1). Reported sensitivities and specificities range from 11% to 82% and 81% to 100%, respectively[4,5,7,9,11e14,30,31] The fairly large differences with the present findings may in part be explained by the defined length of histological follow-up. Other studies may have adhered to a shorter maximum time interval of cytology to histology, thereby selecting those patients with advanced disease where cytological diagnosis may be easier. Determining if a late histological diagnosis explains the ND is hard and arbitrary. However, cancers can grow slowly over years and may still be identified after more than 10 years after clinical presentation.[32] The high number of false-negative diagnoses may be the result of (1) only 1-5% of breast cancers producing ND [26,28] and (2) cancer cells not always being present in ND.[33] Further complicating the matter, the odds of malignancy cannot be reliably estimated on the basis of the degree of atypia, since discharged cells degenerate in the secreting duct.[12] False-positive results are less frequent and may in part result from discharged atypical papilloma cells.[14]

Macroscopic ND examination

In fact, even the examination of macroscopic ND colour alone may be a valuable alternative to ND cytology (Table 3). When bloody ND is considered positive for malignancy, ND colour examination has a remarkably higher sensitivity (60.6% vs. 18.2%, respectively) and only a slightly lower specificity (53.6% vs. 65.0%, respectively) when compared to ND cytology. In the presence of a test that could replace cytology for its specificity, such as physical examination, no single adjunctive benefit of cytology would remain.

Yet, in order to maximize their diagnostic yield, ND colour and cytology are used in conjunction with each other. Although most of the research on the two subjects has considered them separately, one study states that cytology is only useful in the diagnosis of bloody ND.[22] Surprisingly, in the present study cytological examination of bloody ND has a lower specificity than otherwise coloured ND (Table 2). Cytology sensitivity was also lower for bloody ND, but this difference was not significant. This indicates a low relevance of cytology for clinically high-risk ND, mainly complicating diagnostic decision-making in these cases.

Study properties and implications

An acknowledged limitation of this study is its retrospective nature. ND characteristics, such as colour, uni- or bilaterality and spontaneity were obviously subjective. Moreover, these and other variables vary greatly in time in a substantial portion of ND patients. This may also clarify the many contradicting results in this field of research.

Regardless of its retrospectively established (unique) diagnostic accuracy, the role of ND cytology in clinical decision-making is questionable. Of course, clinicians should not rely on cytology alone. However, many authors still regard it as 'useful' in cancer detection. This notion is reflected by the current data as well. Suspect cytological diagnoses tend to be quickly followed by surgery, implying the clinician's urge to obey cytological warnings. However, cytology does not predict surgical outcome (Table 1).

Conclusion

Nipple discharge smearing and its cytological examination have little complementary diagnostic value. Therefore, its routine use for detection of ND-related breast pathology should be reconsidered carefully. Nipple discharge cytology may usefully redirect patient management in some cases, but it may confuse work-up in the majority.

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Chapter 5

Is Cytology Useful in the Diagnostic Workup of Male Breast Lesions? A Retrospective Study Over a 16-Year Period and Review of the Recent Literature

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ABSTRACT

Background

To determine the value of cytology in the workup of male breast lesions, important for the management in a same-day breast clinic.

Methods

A total of 146 fine needle aspirations (FNAs) from the male breast were classified in the categories malignant, suspicious, atypical, benign and inadequate. Cytohistologic correlation was done.

Results

Histologic correlation was available in 85 cases. On FNA the 15 malignant cases were classified as malignant (n = 11), suspicious for malignancy (n = 2) or atypical (n = 2). Of the 35 benign lesions on histology 3 cases were classified as atypia and 1 as suspicious for malignancy on FNA. In the inadequate FNAs (n = 45), the corresponding histologic specimens were benign, no carcinomas were diagnosed. The sensitivity and specificity of the FNA compared to the definite resection diagnosis were 100% and 90.2%, respectively. The results were comparable with the outcomes of the reviewed studies on male breast lesions in the recent literature.

Conclusion

Based on the nature of the benign breast lesions in man, a substantial number of inadequate FNAs were obtained. However, due to the good cytohistologic correlations in the group of malignant lesions, we can conclude that cytology remains an important diagnostic tool in the initial workup of male breast carcinomas.

INTRODUCTION

Fine needle aspiration (FNA) cytology has proven to be a quick, accurate and cost-effective method in the workup of all sorts of tumours from a variety of body sites.[1] Despite the emerging role of the core needle biopsy (CNB), FNA continues to be a useful tool in the initial evaluation of breast masses.[2] However, the core wash or core imprint technique is gaining in popularity, for these techniques generate a quick preliminary cytologic diagnosis, which is important for same-day patient counselling, while the histologic diagnosis follows within 24 hours.

Tumours of the male breast, especially carcinomas, are rare as compared to breast cancer in women. In the Netherlands, the incidence of breast carcinoma in men is 0.9 per 100,000 men as compared with 126.7 per 100,000 women (data from the Dutch Cancer Registry, 2006). The initial workup of a suspicious breast lesion in men is the same as is used for the female breast. In the case of asymmetry or a suspicious mass, FNA cytology is performed.

A limited number of studies have addressed the problems and pitfalls associated with male breast cytology.[3-9] In this study we looked at the possible diagnostic problems that cytology can yield in male breast lesions and how to deal with them. In this regard, we focused on the proportion of nondiagnostic and atypical FNAs as both items preclude proper decision making. Does the moderate to severe atypia often seen in gynecomastia lead to false positive results in the FNA diagnosis?

The results of FNA male breast cytology over the past 16 years in our institute are used in addition to a critical review the literature from 2001.[6,7,9]

MATERIALS AND METHODS

All male breast FNAs, available CNBs and surgical specimens at our laboratory from January 1992 to January 2008 were identified through the national pathology database of the Netherlands. The analyses of the current retrospective study were based on specimens from patients visiting a regional teaching hospital and a regional nonteaching hospital. In both hospitals breast patient care is managed according to national guidelines. Cytologic specimens were obtained by surgeons and radiologists. Either a 21- or a 23-gauge needle was used for aspiration. Before 1997 the aspirate was smeared onto a slide and instantly fixated in ethanol 95% to a conventional smear. After 1997, most FNAs were liquid-based preparations (LBPs). In this cytospin technique the cells were rinsed from the needle and syringe into a vial and directly fixed in an ethanol 50%, polyethylene glycol 2% solution. A cytocentrifuge procedure was applied, creating a monolayer arrangement of all cells within a 12mm diameter area.[8] Both conventional slides and LBP slides were Papanicolaou stained.

The slides were screened by cytotechnologists and examined by 10 staff cytopathologists with 6–20 years of experience in cytopathology. Using simple and well-defined criteria, they classified each aspirate into one of the 5 diagnostic categories proposed by the 1996 National Cancer Institute–sponsored conference approach: malignant, suspicious for malignancy, atypical, benign and inadequate.[10] We calculated the complete sensitivity as positive cases on FNA (atypia, suspicious for malignancy and malignant) divided by positive cases on

histology (carcinoma or ductal carcinoma in situ), the absolute sensitivity as the only malignant cases on FNA divided by the malignant cases on the final histology and specificity as negative cases on FNA (benign) divided by nonmalignant cases on histology. Inadequate specimens were omitted from the accuracy calculation.
The sex was always known to the pathologist at the time of the cytopathologic examination. Further information regarding the clinical presentation of the breast lesion was mostly available to the pathologist. The cytologic diagnoses were correlated with the available histologic findings. The inadequate cases were reviewed independently by 2 pathologists and scored in 4 groups: no material, only blood, only some stromal elements and < 6 epithelial cell clusters. These outcomes were correlated with the available clinical information.
In order to put our figures in perspective, we compared our study with 3 recent published male studies. We performed some recalculations in the 3 studies for valid comparison and included only the cases with available histology; metastatic lesions were omitted.[6,7,9]

RESULTS

From January 1993 to January 2009, a period of 16 years, a total of 8,483 FNAs of the breast were examined. One hundred forty-seven FNAs (1.7%) were from 147 male breasts. They all underwent unilateral FNA cytology. The median age of the patients was 53 years (range, 15–95). The median time interval between FNA and histology was 21 days.
Satisfactory aspirates were obtained in 83 of the 147 cases. These were categorized as benign in 78 cases, atypical in 9 cases, suspicious for malignancy in 3 cases and malignant in 11 cases. Forty-five of the 146 cases had unsatisfactory aspirates, which were not useful for cytologic examination.
Histologic examinations on excisions and biopsy or mastectomy specimens were available in 85 of the 147 FNA cases (Table 1).
Fifteen of the 16 histologically documented cases were primary male breast carcinomas, including 14 infiltrating ductal carcinomas and one apocrine carcinoma. One tumour was a metastatic neuroendocrine lung tumour. The clinical presentation of the primary breast carcinomas suggested in 11 cases a palpable tumour, suspicious for carcinoma, in 1 case a palpable tumour probably benign, 2 cases with a palpable tumour not otherwise specified and one case a lesion suspicious for a cyst. The corresponding FNA diagnosis was malignant in 11 cases, suspicious for carcinoma in 2 cases and atypical in 2 cases. One FNA diagnosis suspicious for carcinoma showed on the slide only a few atypical cells; the other showed proliferating duct epithelium with atypia. In the 2 cases atypical on FNA, 1 tumour of the cutaneous appendages was suggested on cytology, and the other atypical case was the only apocrine carcinoma on histology.

Table1 Correlation of 85 FNA cytology diagnoses with the corresponding histologic diagnoses

	FNA diagnoses				
Histologic diagnosis	Malignant	Suspicious	Atypical	Benign	Unsatisfactory/ Inadequate
Primary breast carcinoma	11	2	2		
Metastasis	1				
Gynecomastia		1	3	25	15
Infection				8	3
Lipoma of fatty tissue				2	3
Fibrosis					4
Others				2	3
Total	12	3	5	37	28

Of the 78 cases reported cytologically as benign, histology was available in 37. All 37 cases were benign lesions, with 25 reported as gynecomastia, 8 cases as infections, 2 cases as lipoma or fatty tissue, 1 case as syringomatous adenoma of the nipple and 1 case of normal tissue on histology.

The 29 cases of gynecomastia on histology had an adequate FNA. The clinical information with the FNA suggested in 4 cases a malignant tumour, in 3 cases a benign tumour, in 16 cases gynecomastia and in 1 case a lipoma. In 5 cases no clinical information was available.

The case suspicious for malignancy on FNA and gynecomastia in the final histologic diagnosis had no clinical data. The clinical information on the 3 cases atypical on FNA suggested in 2 cases gynecomastia, and in 1 case the clinician suspected a malignancy.

Of 45 cases diagnosed as unsatisfactory or inadequate on FNA, 28 had histologic follow-up. The 28 specimens revealed 15 gynecomastia cases, 3 infection cases, 3 lipoma cases or fatty tissue, 4 fibrotic tissue cases, 1 hemangioma, 1 benign cyst and 1 biopsy that was not representative. For the 17 cases without follow-up we checked the national pathology databank; no carcinomas were seen.

The inadequate specimens were reviewed. In 14 cases no cell material was seen, in 15 cases only blood, in 5 cases only some stromal elements and in 3 cases < 6 epithelial cell clusters. Two cases were not available in the archive. The clinical information suggested a malignancy in 7 cases, a benign tumor in 12 cases, in 11 cases there was no clinical judgment about the palpable lesion, a gynecomastia was suspected in 9 cases, an infection in 4 cases, a lipoma in 1 case and a cyst in 1 case (Table 2).

The diagnosis of atypia was reported on FNA 9 times. Five cases had histologic follow-up. Three cases eventually proved to be gynecomastia. In 2 cases a diagnostic CNB revealed breast cancer, followed by a mastectomy.

The absolute sensitivity was 71.4%; the complete sensitivity was 100%. The specificity was 90.2%.

Review of the Recent Literature

Westenend and Jobse[9] included 153 FNAs of the male breast out of a group of 'more than 10,000 FNAs' during a period of 15 years (approximately 1.5%). One hundred forty-one samples were from unilateral lesions. Of all male breast FNAs 9.8% were primary malignant

breast tumours. In the unsatisfactory FNAs (18, 11.7%) no carcinoma or ductal carcinoma in

Table 2 The reviewed 45 inadequate FNAs: correlation between clinical information (vertical) and defined cytologic categories (horizontal) with the available histologic follow-up

	Cytological score									
	No material		Blood		Stromal elements		<6 epithelial cell clusters		No slides	
	No.	Hist.	No.	Hist.	No.	Hist.	No.	Hist.	No.	Hist.
Clinical information										
Malignant tumour (n=7)	3	1 gyn 1 lipoma	2	1 gyn	1	1 infection	1	1 gyn	-	
Benign tumor (n=12)	3	2 gyn 1 cyst	5	1 gyn 1 hg	2	1 gyn 1 not repr.	2	1 gyn	-	
Tumour NOS (n=11)	2	2 gyn	3	2 fibrosis	1	1 infection	3	1 gyn	2	1 lipoma
Gynaecomastia (n=9)	4	2 gyn	5	1 gyn 1 lipoma	-		-		-	
Infection (n=4)	2	1 gyn 1 infection	-		1	1 fibrosis	1	1 infection	-	
Cyst/lipoma (n=2)	-		-				2		-	
Total	14		15		5		9		2	

No., number of cases cytologic categories; Hist., histology; Tumour NOS, tumour not otherwise specified; Gyn, gynecomastia; hg, haemangioma; Not repr., not representative

situ was found. The amount of atypical or suspicious for malignancy cases on FNA with related histology was not mentioned; however, the sensitivity was 100% and the specificity 89% when the inadequate FNAs were excluded (Table 3).

Siddiqui et al[7] reported on 520 diagnostic aspirates from a palpable lump in a male breast out of a total group of 14,026 breast FNAs (male and female, 3.7%) in a period of 10 years. All aspirates of the male breast were from unilateral lesions. The FNAs revealed malignancy in 32 cases. Fifteen cases were breast carcinomas; however, only 14 were primary breast carcinomas (ductal carcinoma not otherwise specified) with corresponding histology. In 170 cases histology was available. Of all male FNAs 6.1% were malignancies; only 2.8% were primary male breast carcinomas. On FNA atypia or suspicious for malignancy was diagnosed in 61 cases. The corresponding histology was available in 57 cases and revealed 7 carcinomas and 2 ductal carcinoma in situ cases. Ninety-four (15.4%) cases were unsatisfactory on FNA; the corresponding tissue diagnosis, available in 21 cases, showed 1 ductal carcinoma in situ and 1 carcinoma. Considering malignant, suspicious for malignancy and atypia on FNA as positive, the calculated sensitivity was 95.3% and the specificity 100%.

MacIntosh et al[6] studied, with 2 groups of observers, the accuracy and reproducibility of FNA on the male breast; this was the third large study on male breast carcinomas since 2001 that we compared. They reported on 138 breast FNAs performed on 123 male patients. One third, 46 FNAs (33.3%), were unsatisfactory. Histologic correlation was available for 23 satisfactory FNAs. Eleven cases were primary breast carcinomas on histology. The corresponding FNAs were malignant in 7 cases and suspicious for malignancy in 4 cases. The

12 benign cases showed on FNA unremarkable or proliferative characteristics in 11 cases and atypical in 1 case by 1 group of observers; the other group considered all corresponding FNAs unremarkable or proliferative. The sensitivity was 95.5% and the specificity 100%.
The sensitivity calculated in the 3 studies was only the complete sensitivity; the absolute sensitivity was not available.

DISCUSSION

In this study, 1.7% of all breast FNAs were from the male breast. In all large cytologic studies of breast lesions, FNA on the male breast constitutes 1.54–3% of the total cases.[3-9] The majority of male breast lesions turned out to be benign. Although the incidence of male breast carcinomas is increasing, it still remains a very rare condition as compared to the incidence in females.[3-9,11] In our study 10.2% of all male breast FNAs were primary malignant tumours; one lesion was metastatic. The complete sensitivity and the specificity of FNA as compared to the definitive resection diagnosis were 100% and 90.2%, respectively.

Table 3 Results of this study in comparison with the studies by Westenend and Jobse, Siddiqui et al and MacIntosh et al

Result	2002 Westenend	2002 Siddiqui	2008 Macintosh	2009 this study
No. of male FNAs (% male FNAs of all breast FNAs)	153 (1,5%)	614 (4,3%)	138 (3,2%)	147 (1,7%)
No. of cases with histologic follow-up	72	170	23	85
No. of malignant cases (% primary breast carcinomas)	15 (9,8%)	26 (2,8%)	11 (7,9%)	15 (10,2%)*
No. of unsatisfactory cases of all FNAs	18 (11,7%)	94 (15,4%)	46 (33,3%)	45 (30,6%)
No. of unsatisfactory FNAs with histologic follow-up	10**	21**	***	28
Sensitivity	100%	95,3%	95,5%	100%
Specificity	89%	100%	100%	90,2%

*The metastases were excluded from the calculations.
**In both studies inadequate FNAs revealed 2 carcinomas in follow-up.
***The unsatisfactory or inadequate cases were not discussed in this study

Because the last review of the literature on male breast lesions dates from 2001, we compared our results with those of 3 large studies since 2002[6,7,9] (Table 2). In line with these studies we considered atypia, suspicious for malignancy and malignant as a positive result and benign as a negative result. In order to compare the data, it was necessary to draw 1 line although we were aware that the further workup of the breast lesion is different for atypia and suspicious for malignancy than for the FNA diagnosis malignant. After all, the latter result does not need a complementary CNB.
Our study and the study by Westenend and Jobse[9] showed the highest percentage of primary breast carcinomas diagnosed on FNA cytology, 10.2% and 9.8%, respectively. Siddiqui et al[7]

had a percentage of only 2.8. As shown in Table 2, the sensitivity ranged from 89.7% to 100%. The specificity of 90.2% in our study was due to 4 false positive FNA diagnoses on gynecomastia.

Gynecomastia is the most common cause of masses in the male breast and is defined as the enlargement of the male breast due to proliferation of both glandular and stromal elements.[12] According to the literature and the cytologic textbooks, gynecomastia may present on FNA with dyshesive groups and sheets of ductular cells with moderate to severe nuclear atypia.[3,8] Because of these cytologic criteria, one should expect many cases of atypia on FNA. Westenend and Jobse[9] mentioned < 1% atypia, and the other 2 studies did not report the frequency of atypia as a diagnostic group. In our study, atypia was diagnosed in 11 cases on FNA. Five cases had histologic follow-up. Two cases were carcinomas on histology, and 3 were gynecomastia.

Our study showed 44 cases of gynecomastia. One case was diagnosed as suspicious for malignancy, and in 3 cases the diagnosis atypia was reported on FNA. Malignant was never the FNA diagnosis in cases of gynecomastia.

So, in contrast to what was expected, we can conclude that in practice the diagnosis gynecomastia did not give much of a challenge on FNA. However, gynecomastias render a substantial number of unsatisfactory or inadequate cases on FNA. As shown, 15 of the 44 cases were inadequate (Table 2). In line with other authors who also had this high percentage of unsatisfactory cases, we think it is due to the nature of the lesion, gynecomastia, and not to the FNA technique itself.

All studies showed many unsatisfactory cases, ranging from 11.7% to 33.3%.[6,7,9] In our study we had 45 (30.6%) unsatisfactory cases on FNA. However, in the clinical presentation a malignant lesion was suspected only 7 times. Twenty-eight cases had histologic followup, and no carcinomas were observed. The unsatisfactory cases in the studies by Westenend and Jobse[9] and Siddiqui et al[7] both had 2 carcinomas on histology. MacIntosh et al[6] did not discuss unsatisfactory or inadequate samples.

or the last decade, CNB has been introduced and for several reasons is preferred in the workup of breast lesions. However, in order to obtain a quick preliminary diagnosis, essential for the management of the 1-day breast clinic, core wash or touch imprint cytology of the CNB can be done, for it can provide a diagnosis within half an hour. However, the interpretation of the slides obtained by the wash or imprint techniques are still based on the cytologic criteria of the FNA. In this respect, we can combine the benefits of both modalities in the future.

Conclusion

Despite not yielding a conclusive diagnosis in a substantial number of cases, the high sensitivity and specificity justifies primarily opting for cytology in the initial diagnostic workup of male breast lesions.

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Chapter 6

Modified Core Wash Cytology Procedure for the Immediate Diagnosis of Core Needle Biopsies of Breast Lesions

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ABSTRACT

Background

Core wash or touch imprint cytology is often used to obtain a quick, preliminary diagnosis on a core needle biopsy (CNB) of breast lesions, essential for the management of the 1-day breast clinic. Contradictory results of both techniques in the literature led to this preclinical study investigating an alternative method of touch imprint and core wash cytology.

Methods

Thirty breast lesions were biopsied by a core needle in a laboratory setting. The CNBs were collected in RPMI fluid (Roswell Park Memorial Institute fluid). The touch imprint cytology was performed taking the biopsy out of the fluid and smearing it on a microscopic slide and May-Grunwald Giemsa stained. The core wash cytology was made by fixating the remaining cells in Fixcyt and prepared with a liquid-based preparation method and Papanicolaou stained. The cytologic findings were categorized into benign, atypical favoring benign, atypical, suspicious, and malignant and compared with the histologic CNB results.

Results

The CNBs showed 20 of 30 samples to be malignant, 2 to be phylloides tumours, 7 to be benign, and 1 to be unsatisfactory. Both techniques showed a sensitivity of 95% and specificity of 100%. Touch imprint yielded insufficient diagnoses (13.3%), compared with core wash (6.6%). Of the core wash cases, 86% showed a good quality versus 30% in touch imprint cytology.

Conclusion

This preclinical study on modified touch imprint and core wash techniques led to results that were comparable to or better than those in the literature. The core wash cytology is preferred to touch imprint because of the better morphology.

INTRODUCTION

Fine-needle aspiration (FNA) cytology is frequently used in the instant diagnosis of breast lesions. The histologic diagnosis, however, gains importance in the initial diagnostic workup. A core needle biopsy (CNB) is often preferred to cytology because it can provide more information about the tumour characteristics, precise type of carcinoma, and hormonal and other receptor status.

However, CNB diagnosis requires 24 hours of processing whereas cytology can allow diagnosis within 1 hour.[1] A quick reliable preliminary diagnosis is very important for same-day patient counselling and expedites the planning of further surgical or neoadjuvant management. Immediate diagnosis of breast lesions is also useful for alleviating patient anxiety. Most patients prefer to receive their results on the same day as their tests.[2-6]

Core wash in a saline solution or touch imprint cytology could be an answer to this problem. There are several studies about touch imprint cytology derived from CNBs in breast lesions. The sensitivity and specificity vary from 74%-100% and 83.7%-100%, respectively.[1-4,7-9] The described procedures, however, result in a substantial proportion of unsatisfactory samples.

It was recently stated that core wash cytology was not useful at all for the immediate diagnosis of breast lesions by ultrasound-guided CNB.[6] A sensitivity and specificity of 89% and 75%, respectively, was shown with a 42% unsatisfactory rate.

In trying to integrate a rapid preliminary diagnosis with CNB, both core wash and touch imprint techniques were modified. The modified core wash and touch imprint procedures were tested in an in vitro laboratory setting. The cytologic results were correlated to the CNB histology.

MATERIALS AND METHODS

In a laboratory setting, 30 lesions in 27 surgical resected breast specimens (lumps, mastectomies) were biopsied before fixation by 1 cytologic technician or 1 pathologist. Two to 3 CNBs per lesion were obtained in 18 in vivo palpable and 12 nonpalpable breast lesions with an 18-gauge needle (Bard Needle Monopty, eGeneral Medical Inc., Murray Hill, NJ) and biopsy gun. No ultrasound guidance or other imaging techniques were used.

Unique and in contrast to other preparation manuals, the biopsies were collected in 6 mL RPMI medium (Roswell Park Memorial Institute, Invitrogen, Breda, the Netherlands). To each 500 mL, 0.5 grams Natrium-azide (Sigma-Aldrich Chemie B.V., Zwijndrecht, the Netherlands) and 5 grams of bovine serum albumin were added. The biopsies stayed in this mixture for 5-10 minutes. The needle tip was rinsed in the medium.

The touch imprint cytology preparations included carefully taking the biopsy out of the medium and smearing it on a microscopic slide. The touch imprint slides were air dried and May-Grunwald Giemsa stained.

Table1 Explanative terminology of core wash and touch imprint cytology diagnosis categories

Core Wash/Touch Imprint Diagnosis	Positive/Negative	Adequacy
Inadequate	-	Inadequate
Benign	Negative	-
Atypia favouring benign	-	-
Atypia	-	Adequate
Suspicious for carcinoma	Positive	-
Malignant	-	-

After this, the biopsy was fixated in formalin, paraffin embedded, and H&E stained.
The core wash cytology preparations included fixing the remaining cells in the medium for 10 minutes in 2 mL Fixcyt (50% ethanol and 2% polyethylene glycol solution). According to the operator manuals, this mixture was centrifuged for 10 minutes at 688 rpm in a Hettich Rotina 48S or Hettich Rotanta 46S centrifuge (Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany). Supernatant was discarded. Depending on the estimated density of the sediment, 1 or 2 drops of the ethanol 50% and polyethylene glycol 2% solution were added. The resultant mixture was dripped into a bucket that was clasped on a polysine slide (Menzel GmbH, Braunschweig, Germany). By centrifuging for 5 minutes at 688 rpm, the sediment was pressed onto the slide, creating a monolayer arrangement of all cells within a 12 mm diameter area.[10] The liquid based preparation slides were Papanicolaou-stained.
To quantify the quality of the cells, we scored the morphology of the cells into 3 categories: poor, not optimal, and good morphology. In the case of poor morphology, no diagnosis could be made. In the case of suboptimal morphology, it was hard to make a diagnosis but possible. With the good morphology case, a diagnosis was easy to make.
The cellularity was also divided in 3 groups: poor (<6 epithelial cell clusters), moderate (6-10 epithelial cell clusters), and rich (>10 epithelial cell clusters). The presence of more than 6 epithelial cell clusters was required for adequate classification.
According to international guidelines for FNA cytology, the core wash and touch imprint cytological findings were categorized as follows: benign, atypical favouring benign, atypical, suspicious for carcinoma, malignant, or inadequate (Table 1). On CNB histologic diagnosis, we considered carcinoma, suspicious for carcinoma, and ductal carcinoma in situ (DCIS) as positive for carcinoma. 'Not representative' and 'no material' were put in the unsatisfactory category.
Two experienced observers read the CNB histology and the core wash and touch imprint slides in a blinded fashion. The results of the core wash and touch imprint cytology were correlated with the diagnosis of the CNB. Sensitivity was defined as the proportion of positive and suspicious core wash or touch imprint cytology cases in malignant lesions (which include DCIS, carcinoma, and suspicious for carcinoma) on CNB histology.
Specificity was defined as the number of negative core wash or touch imprint cytology cases divided by the number of positive and negative cytology specimens with subsequent benign histology on CNB. 'Not representative' and 'no material' were put in the unsatisfactory category and left out of the calculations.

RESULTS

The CNBs were histologically diagnosed as carcinoma (n = 17), DCIS (n = 1), suspicious for carcinoma (n = 2), atypical stroma/phylloides tumour (n = 2), benign (n = 7), and not representative/normal breast tissue (n = 1). The benign category (n = 7) comprised fibroadenoma (FA; n = 1), FA/fibrotic breast tissue (n = 2), fibrotic breast tissue (n = 3), and sclerosing adenosis (n =1).

The core wash corresponding to the 20 malignant CNBs were cytologically diagnosed as malignant in 15 cases (75%), suspicious in 4 cases (20%), and atypia favouring benign in 1 case (5%) (Table 2). The atypia case on core wash was suggestive of carcinoma on CNB.

Table 2 Correlation of diagnoses of core wash cytology and coordinating core needle biopsy histology

	Core Needle Biopsy Histology			
Cytologic Diagnosis	Positive for carcinoma	Phylloides	Benign	Unsatisfactory
Carcinoma	15	-	-	-
Suspicious	4	-	-	-
Atypia favouring	1	2	6	-
Unsatisfactory	-	-	1	1
Total	20	2	7	1

All atypical cases diagnosed in core wash were atypia favouring benign. The phylloides tumours were diagnosed as atypia on core wash with the remark of stromal atypia. The sclerosing adenosis case was diagnosed as atypia on core wash. The FA in the CNB showed atypia consistent with FA on core wash. In 2 cases, it was not possible to differentiate between FA and fibrotic breast tissue on CNB. The corresponding core wash revealed 1 unsatisfactory and 1 atypia specimen. Fibrotic breast tissue on CNB, 3 cases, was once diagnosed as unsatisfactory and twice as atypical on core wash. The sensitivity was 95% and the specificity was 100%.

The touch imprint corresponding to the 20 histological diagnosed carcinomas comprised 15 carcinomas (75%), 3 suspicious (15%), 1 atypia favouring benign (5%), and 1 unsatisfactory (5%) (Table 3). The phylloides tumours were also diagnosed as stromal atypia. The CNB diagnosis of sclerosing adenosis showed atypia on touch imprint. The FA on CNB revealed atypia on touch imprint, FA/sclerosing adenosis was in the touch imprint 2 times unsatisfactory, and fibrotic tissue was in touch imprint once unsatisfactory and twice atypia. Normal breast tissue on CNB showed atypia on touch imprint. The atypia seen in touch imprint was atypia favouring benign. The sensitivity was 95% and the specificity was 100%.

Core wash and touch imprint showed almost the same results in cellularity. It was poor in 2 core wash (7%) cases and in 3 touch imprint (10%) cases, moderate in 3 core wash (10%) cases and in 2 touch imprint (7%) cases. Twenty-five cases (83%) showed a rich cellularity in both core wash and touch imprint techniques.

Table 3 Correlation of diagnoses of touch imprint cytology and coordinating core needle biopsy histology

	Core Needle Biopsy Histology			
Cytologic Diagnosis	Positive for carcinoma	Phylloides	Benign	Unsatisfactory
Carcinoma	15	-	-	-
Suspicious	3	-	-	-
Atypia favouring	1	2	4	1
Unsatisfactory	1	-	3	-
Total	20	2	7	1

The morphology was poor in 2 core wash cases (7%) and in 4 touch imprint cases (13%). Although not optimal, a diagnosis could be made in again 2 core wash cases (7%) and in 17 touch imprint cases (57%). The morphology was good in 26 core wash cases (86%) and in 9 touch imprint cases (30%) (Table 4).

Table 4 Quality morphology in core wash and touch imprint

Quality	Core Wash	Touch Imprint
Poor	2	4
Moderate	2	17
Good	26	9
Total	30	30

DISCUSSION

In the initial diagnostic workup, the CNB yields more essential information about the tumour. However, an evaluation of the tissue sample requires 24 hours of processing. A rapid diagnostic method to cytological analysis of the CNB can offer a diagnosis in 1 hour. Only for the price of a touch imprint or core wash derived from a CNB, the clinician will be informed about the nature of the breast lesion and he will be able to plan further treatment the same day.

By using a modified processing technique for obtaining cytology from CNB, sensitivity and specificity for both core wash and touch imprint were 95 and 100%, respectively. The unsatisfactory rate was 6.6% in the core wash and 13.3% in the touch imprint cytology. In contrast to the literature, the malignant tumours were never unsatisfactory in our study.[5-7]

The diagnostics accuracies of touch imprint and core wash cytology are conflicting in the literature.

Some authors reported a sensitivity and specificity of 85% and 98%, respectively, in their clinical study with core wash cytology, using a 16-gauge core needle.[5] They had an unsatisfactory rate of 7% and regarded the core wash technique as useful for immediate diagnosis.

Others reported a sensitivity of 89% and a specificity of 72% of core wash cytology.[6] The unsatisfactory rate in this study was 42%. A quarter of these unsatisfactory core wash results

had a malignant diagnosis on CNB. The procedure was slightly different and they used an 18-gauge core needle. The study was done in a clinical setting. They concluded that the core wash technique was not useful for quick cytological diagnosis on CNB. A sensitivity and specificity of 100% each was seen in a study using an 11-gauge core needle.[2]

Our figures can compete with these studies. However, we are aware that about 67% of the lesions in our study were malignant. We even did not have to worry about patients' discomfort. But, to resemble a clinical setting, we took only 2 or 3 CNBs from each breast lesion. The studies performed in the clinic had the advantage of ultrasound-guided CNBs. In our study, no ultrasound guiding or other imaging techniques were used. The nonpalpable lesions were biopsied randomly.

Touch imprint cytology of the CNB has a more widespread use than the core wash. Some authors use it for evaluation of the quality of the CNB only.[8] Others use the technique to investigate the possibility of needle tract seeding.[11] Still, touch imprint is mostly used for providing a preliminary diagnosis. The sensitivity ranged from 74%95%, whereas specificity ranged from 97%-100%.[1-4,7,9]

As in the literature, we also had problems in classifying the benign lesions in the cytology.[1] Possibly, this is caused by the characteristics of these lesions. In benign tumours the cohesiveness of the tissue and cells is much higher than in malignant lesions.[7,11] In our study, the histological benign diagnosis on touch imprint or core wash cytology was noticed as benign or atypia favouring benign.

Our study showed slight differences between core wash and touch imprint in figures. The unsatisfactory rates were 6.6% and 13.3%, respectively. The core wash was unsatisfactory in one benign and one unsatisfactory diagnosis on CNB. Touch imprint was tree times unsatisfactory on benign histology and once on suspicious for carcinoma on CNB. However, we are aware that we biopsied a small number of lesions.

The better cytological results in both the core wash and the touch imprint cytology in our laboratory could probably be attributed to the fact the CNB is collected in RPMI medium. The RPMI medium is used for the culture of human normal and neoplastic leukocytes. It has demonstrated wide applicability for supporting growth in many types of cultured cells. Therefore, it is a good medium in which to preserve the loosened cells optimally. This feature is in contrast to saline, which causes swelling of the cells and often leads to bursting of the cells.[5,11]

The better morphology seen in core wash (Table 4) is probably because of the finding that the cell material is worked up in a standardized procedure that collects all cells in the fluid and that is not operator-dependent.[10] The quality of the touch imprint cytology is highly dependent on the manual skills of the person performing the biopsy and making the touch imprint slides.

Another known advantage of core wash cytology is the microscopic evaluation. The cells are pressed in a monolayer arrangement within a 12 mm diameter area, whereas the microscopic screenings area with touch imprint cytology is the whole slide.

All these arguments turned the scale, in our practice, in choosing the core wash procedure at expense of touch imprint.

Conclusion

In conclusion, according to our findings the modified core wash procedure can provide a reliable preliminary indication of the CNB results. This information is valuable for same-day patient counselling and management planning.

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Chapter 7

Modified core wash cytology (CWC), an asset in the diagnostic work-up of breast lesions

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ABSTRACT

Background

A quick and reliable preliminary diagnosis is essential in the management of a same-day breast clinic. In a preclinical study we developed an alternative method of core wash cytology (CWC). This study is an evaluation of this new CWC method introduced into the clinical setting.

Methods

From April 2008 to April 2009, biopsies were taken from lesions in the breast. CWC was obtained from core needle biopsy (CNB) with a modified technique and classified into the categories: malignant, suspicious for malignancy, atypical, benign and inadequate. CWC and CNB diagnoses were correlated with the histopathology of subsequently obtained resection specimens. The sensitivity and specificity were calculated.

Results

CWC was obtained from 226 breast lesions. In 167 of these cases subsequent resection of the lesion was performed revealing 149 carcinomas and 18 benign lesions. Of the 149 malignant cases, 136 were considered as either malignant or suspicious for malignancy by CWC, 7 as atypical, 4 as benign and 2 as inadequate. None of the 18 benign lesions were classified as suspicious or malignant on CWC. Eight out of 149 resected carcinomas were not recognized as malignant by histological analysis of the CNB, while 7 of these cases the CWC was considered malignant. The sensitivity and specificity were 97% and 100%, respectively.

Conclusion

In the vast majority of patients the modified CWC technique can provide a quick and reliable diagnosis of malignant breast lesions. Furthermore, combining CWC with CNB histology can improve adequate, preoperative recognition of the malignant character of breast lesions.

INTRODUCTION

The core needle biopsy (CNB) is increasingly being used as a first-line diagnostic modality in the diagnostic work-up of palpable and non-palpable breast lesions. Indeed, histological examination of such biopsies generally provides very important and detailed information about tumour characteristics, including subtype of carcinoma, hormonal status and other molecular features.

However, a same-day breast clinic requires a quick and reliable diagnosis for same-day patient counselling and optimal planning of further surgical or neo-adjuvant management. In most laboratories, a CNB diagnosis takes 12-24 h of processing. Core wash cytology (CWC) or touch imprint cytology (TIC) could be a solution for this problem because by cytological analysis a diagnosis can often be rendered within 1 h. Unfortunately, the results described in the literature with such cytological diagnostic approaches are variable. In studies on CWC the sensitivity and specificity varied from 85% to 89% and 72% to 98%, respectively, while the inadequate rate varied from 7% to 42%.[1,2] Studies on TIC showed variable results as well.[3-10]

We developed a modified CWC technique in which the collecting fluid is optimized and the processing is standardised. This technique was first tested in a laboratory setting and yielded very promising results, especially in malignant lesions.[11] We then introduced the CWC approach into the clinical setting.

In this manuscript we present the results obtained in the first year after the introduction of the modified core wash technique.

MATERIALS AND METHODS

Patient and tissues samples

From April 2008 to April 2009, CNBs were taken from palpable and non-palpable lesions in the female breast in our teaching hospital. According to the Breast Imaging Reporting and Data System (BI-RADS), developed by the American College of Radiology, biopsies were taken by the radiologist. Two to 3 CNBs per lesion were obtained by radiologists, using an 18-gauge needle (Bard Peripheral Vascular, Inc., Tempe, Arizona USA) and biopsy gun under ultrasound guidance.

CWC technique

The biopsies were collected in 6 ml RPMI® medium (Roswell Park Memorial Institute, Invitrogen, Breda, The Netherlands). To each 500 ml RPMI 0.5 g Natrium-azide and 5 g Bovine Serum Albumin (SigmaeAldrich Chemie BV, Zwijndrecht, The Netherlands) were added. The biopsies were kept in the medium for 5-10 min, and additionally the needle tip was rinsed in the medium. Subsequently, the needle biopsies were removed from the medium by a cytological technician, fixed in formalin, paraffin embedded and haematoxylin and eosin stained (Fig. 1).

The CWC was performed by fixing the remaining cells in the medium for 10 min in 2 ml Fixcyt® (50% ethanol/ polyethylene glycol 2% solution). According to the operator's manuals, this mixture was centrifuged for 10 min at 688 rpm in a Hettich Rotina 48S®or

Hettich Rotanta 46S® centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Next, the supernatant was discarded¨ and dependent on the estimated density of the sediment, 1 or 2 drops of the Fixcyt® solution was added. The resultant mixture was collected in a container and then put on a poly-L-lysine slide (Menzel GmbH, Braunschweig, Germany). By centrifuging for 5 min at 688 rpm, the sediment was pressed onto the slide, creating a monolayer arrangement of all cells within a 12 mm diameter area.[12,13] The liquid based preparation slides were Papanicolaoustained (Fig. 1). The preparation and the screening of the CWC slides were done by a cytotechnologist and supervised by a cytopathologist (total group in our centre consisting of 6 cytotechnologists and 8 pathologists with ample experience in cytopathology).
The clinician was informed about the CWC diagnosis within 1 h after puncture and about the histological diagnosis on CNB material early in the following morning.

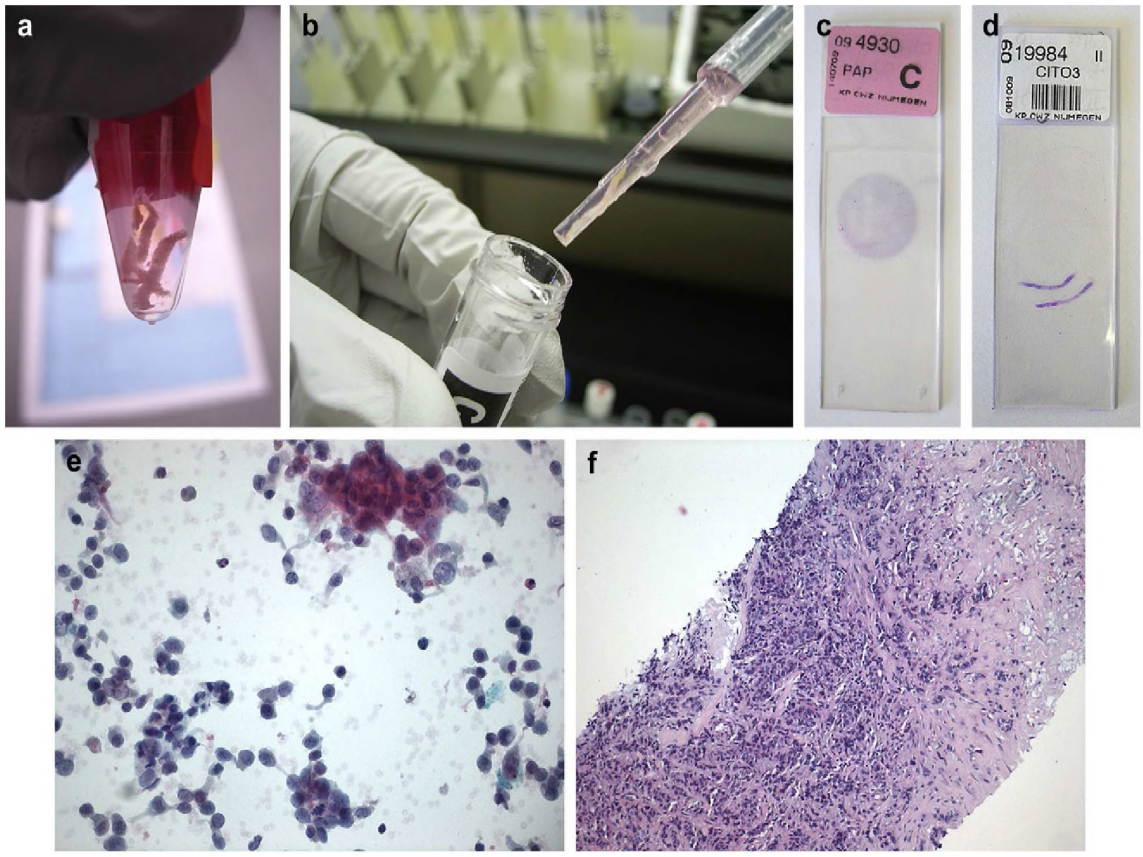

Figure 1 Core wash cytology (CWC) procedure. After collecting the needle biopsy specimens in preservation fluid (a), the tissue fragments are gently rinsed in a syringe (b); subsequently, the tissue fragments are removed, the remaining fluid is centrifuged, and its cytological content deposited on a glass slide and stained for microscopical examination (c); the needle biopsy specimens are routinely processed for histological analysis (d); examples of the microscopy in a CWC sample (e) and of the accompanying needle biopsy specimen are depicted in resp. (e) and (f) (case of ductal breast cancer; original magnification in (e) x 200, in (f) x 200).

Classification

The cytological criteria for CWC are exactly the same as those used for fine needle aspiration (FNA). The presence of more than 6 epithelial cell clusters was required for adequate classification.

According to international guidelines for FNA cytology, the CWC findings were categorized as benign, atypical, suspicious for malignancy, malignant or inadequate. Based on our preclinical study, on CNB, we considered carcinoma, suspicious for carcinoma and ductal carcinoma in situ (DCIS) as 'positive for carcinoma'.[11] Not representative and no material were put in the inadequate category.

The results of the CWC were correlated with the histopathology of the CNB and, when available, subsequent resection specimens. The CWC test was considered as 'conclusive' when the diagnosis on CWC was malignant, suspicious for malignancy or benign. Sensitivity was defined as the proportion of malignant, suspicious for malignancy CWC cases divided by malignant (DCIS, suspicious for carcinoma and carcinoma) cases on the final resection specimen. Specificity was defined as the number of benign CWC cases divided by non-malignant cases on histology of the final resection specimen.

If the CWC diagnosis was malignant or suspicious for malignancy, the patients were planned for further surgical or neo-adjuvant management.

RESULTS

Patients and pathological findings

In a one year period (April 2008 - April 2009), CWC was obtained from the CNB of 226 breast lesions. The age of the patients ranged from 21 to 92 years (median 57,5 years). In 167 of these cases subsequent resection of the lesion was performed in the follow-up.

Table 1 Core wash cytology (CWC) diagnoses and core needle biopsy CNB histological diagnosis (*in italics*) in relation to the final histological diagnoses of malignant lesions in resection specimens.

Final malignant diagnoses	Malignant/suspicious for malignancy	Atypia	Benign	Inadequate
Ductal carc. n = 111	105/*107*	4/*0*	1/*1*	1/*3*
Lobular carc. n = 24	18/*24*	3/*0*	2/*0*	1/*0*
DCIS n = 7	6/*3*	0/*0*	0/*0*	0/*0*
Other malignant lesions n = 7	7/*7*	0/*0*	0/*0*	0/*0*
Total n = 149	136/*141*	7/*0*	4/*1*	2/*7*

Malignant cases

The resection specimens revealed 149 carcinomas (ductal n = 111, lobular n = 24, DCIS n = 7 (one grade 2 and 6 grade 3), mucinous n = 4, tubular n = 1, medullary n = 1, papillary n = 1).

Of the 149 malignant cases, by CWC 136 cases were classified as either malignant (n = 110) or suspicious for malignancy (n = 26), 7 as atypical, 4 as benign and 2 as unsatisfactory (Table 1). Of the 111 cases of ductal carcinoma in the final specimen histology the CWC was

'positive' in 105 cases (malignant n = 92; suspicious for malignancy n = 13), atypical in 4 cases, benign in one case, and inadequate in one case. In 4 of these 111 cases the CWC diagnosis was malignant and the CNB diagnosis was inadequate (n = 3) or benign (n = 1). In the group of 24 lobular carcinomas CWC was 'positive' in 18 cases (malignant n = 11; suspicious for malignancy n = 7), atypical in 3 cases, benign in 2 cases, and one case was non-diagnostic due to inadequate cytological material. In this lobular carcinoma group the histological CNB diagnosis was malignant in all cases.
In the group of 7 cases with DCIS, the final histology revealed one case with ductal carcinoma in situ grade 2, which was suspicious for malignancy on CWC and inadequate on CNB. Of the 6 DCIS grade 3 cases, one case revealed a benign diagnosis on CWC with insufficient diagnostic tissue on CNB. Two of three malignant cases on CNB were also malignant on CWC, one was suspicious for malignancy on CWC. However, two cases revealed the diagnosis suspicious for malignancy on CWC but the CNB was inadequate histological material and did not allow for a diagnosis on CNB.
The 7 mucinous, medullary, tubular and papillary carcinomas were all 'positive' on CWC (malignant n = 5, suspicious for malignancy n = 2) and CNB.
For confirmation of the CWC diagnosis in one of the 4 ductal carcinoma cases another (second) CNB was taken and in all DCIS cases.

Table 2 Results core wash cytology (CWC) diagnosis and core needle biopsy (CNB) (*in italics*) relation to the final benign diagnoses in resection specimens.

Final benign diagnoses		Malignant/suspicious for malignancy	Atypia	Benign	Inadequate
Adenosis	n = 4	0/*0*	2/*0*	0/*4*	2/*0*
Fibrous tumours	n = 9	0/*0*	4/*1*	4/*7*	1/*1*
Intracystic papilloma	n = 1	0/*0*	1/*0*	0/*1*	0/*0*
Others	n = 4	0/*0*	0/*0*	4/*4*	0/*0*
Total	n = 18	0/*0*	7/*1*	8/*16*	3/*1*

Benign cases

Eighteen resected breast lesions were benign including 4 cases of adenosis, 9 benign fibrous tumours, one intracystic papilloma, one case of mastitis, 2 of fat necrosis, and one of mammary duct ectasia (Table 2). On CWC, none of these benign lesions were classified as suspicious for malignancy or malignant, 8 lesions were classified as benign, 7 as atypical, and 3 as inadequate.
The group of 9 fibrous tumours consisted of 6 fibroadenomas which were diagnosed as benign in 3 cases, and as atypical in 3 other cases on CWC. Two of the fibrous tumours were phyllodes tumours on final histological analysis, one case was diagnosed benign in both CWC and CNB histology and in the other case a diagnosis of atypia was given in both techniques. The ninth fibrous tumour was a desmoid fibromatosis which was diagnosed benign on CNB and was inadequate on CWC.

The intracystic papilloma was diagnosed benign on CNB, however showed atypia on CWC. The cases with fat necrosis, mastitis and duct ectasia in the final specimen histology were benign in both the CWC and CNB histology.
The CWC test was considered as 'conclusive' (malignant, suspicious for malignancy or benign) in 88% of the cases. The sensitivity and specificity of the CWC diagnosis compared to the definite resection diagnosis was 97% (95% confidence interval: 93%-99%) and 100% (95% confidence interval: 59%-100%), respectively. The percentage of CWC examinations resulting in the diagnosis inadequate was 4.0%.

Cases without immediate biopsy or resection
In 59 cases no subsequent resection was performed. (Table 3) In 26 cases the CNB was malignant and in 33 cases benign. Of these 26 malignant cases, by CWC 24 cases were classified as either malignant (n = 21) or suspicious for malignancy (n = 3), and in 2 cases atypia was suggested on CWC.

Table 3 Results of core wash cytology (CWC) and core needle biopsy (CNB) of the 59 cases with only 'archival' follow-up confirmation of the diagnosis

	CWC				CNB			
Final diagnosis	Malignant/ suspicious	Atypia	Benign	Inadequate	Malignant	Atypia	Benign	Inadequate
Malignant (adjuvant therapie) n = 15	15/0	0	0	0	15	0	0	0
Malignant (elderly) n = 10	6/3	1	0	0	10	0	0	0
Lymphoma n = 1	0/0	1	0	0	1	0	0	0
Benign n = 33	0/0	7	23	3	0	0	30	3
Total n = 59	21/3	9	23	3	26	0	30	3

Fifteen of these patients with malignant tumours without subsequent resection of the breast lesion underwent neo-adjuvant chemotherapy (mean age 45.6 years), 10 cases received hormonal therapy (mean age 83.6 years in this subgroup), and one patient had a diffuse large B-cell Non Hodgkin lymphoma (which was diagnosed as atypia on CWC) and received chemotherapy.
In none of the 33 cases with a benign CNB diagnosis a CWC diagnosis malignant or suspicious for malignancy was rendered. In these 33 cases, follow-up for pathological diagnosis by the Dutch Network and National Database for Pathology (PALGA), at least 6 months after the CNB still did not reveal malignancy, supporting the benign nature of the breast lesion in these 33 patients.

DISCUSSION

Management breast clinic

For optimal organization of a same-day breast clinic a quick and reliable pathological diagnosis is warranted. A histological diagnosis in 1 h, at all times of the day, would be the best but is until today not available. Touch imprint cytology (TIC) or core wash cytology (CWC) could be a solution in a same-day breast clinic, but variable results were described for both techniques in the literature. A recent study on TIC revealed a sensitivity and specificity of 97.7% and 94.2%, respectively.[10] Interestingly, in that study the inadequate cases (5%) were also excluded from the calculations, but in contrast to our study the cytological diagnosis atypia was included in the benign group. Our study shows that in case of the cytological diagnosis atypia further examination is needed to rule out malignancy.

Modified CWC technique

In a preclinical study we obtained very promising results with a modified CWC technique generating highly informative cytological material from CNBs.[11] Therefore, we introduced this modified technique in the clinical setting. Both in the preclinical and clinical study, the specificity of a 'conclusive' CWC diagnosis was 100%. The sensitivity for this diagnosis was in this clinical study 97% and in the preclinical study 95%, respectively. The percentage of cases with a CWC diagnosis inadequate was comparable in the clinical and preclinical study (4.0% vs. 6.6%). As we stated in the preclinical study, it is important to note that the quality of the results on CWC are for a major part determined by factors such as the experience of the pathologists, the nature and the quality of the collecting medium, the standardised handling of the material by the technicians, and the cytospin monolayer preparation.[3,11,13]

Literature CWC

The recent CWC study of Uematsu et al. on 458 consecutive patients with breast lesions showed a sensitivity and specificity of 89 and 72%, respectively, while 42.2% of the samples were considered as inadequate.[2] Also in that study inadequate samples were more often obtained from benign breast lesions which is probably due to the nature of these lesions. One older study describes similar diagnostic results: 7% inadequate samples on CWC and a sensitivity and specificity of 85 and 98%, respectively.[1] Of note, Lankford et al. used a larger (16-gauge) core needle, while the 18-gauge needle we used may be more comfortable for the patient

Findings from this study

Our present clinical study confirms the observations of our preclinical study.[11] In the malignant group, seven cases were classified as atypical on CWC (4 of 111 ductal and 3 of 24 lobular carcinoma cases). In these cases the biopsies were generally very short and/or fragmented, and a smaller volume of biopsy material may very well result in a lower number of cells available for cytological diagnosis. Also, the percentage of the diagnosis atypia was significantly higher in the group of lobular carcinomas than in ductal carcinoma cases. This might be explained by the fact that lobular carcinoma cells often show only discrete nuclear atypia and can also mimic lymphocytes in cytological specimens. Cells of lobular carcinomas

may therefore be more difficult to recognize as malignant cells, especially when few cells are available for cytological examination.
The 4 cases that were (also retrospectively) classified as benign on CWC and as malignant on CNB all had relatively few cells available for cytological examination but did meet the requirements for adequate material.

Extra diagnostic modality
Interestingly, in 8 cases with a final diagnosis of malignancy in the resection specimen, the histological examination of the CNB did not reveal malignancy. Of these, 4 ductal carcinoma

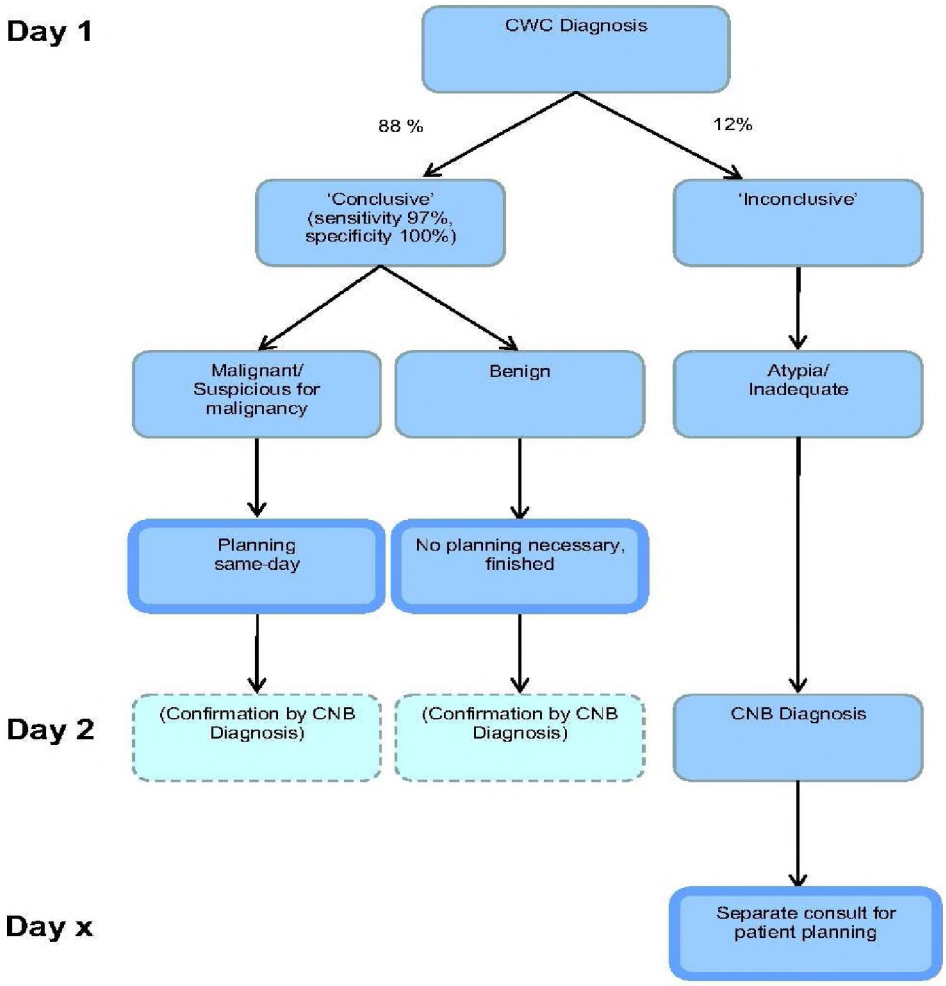

Figure 2 Diagram showing how in the present study the core wash cytology (CWC) diagnosis (because of its high sensitivity and specificity) could be used for same-day counselling and management decisions in the vast majority (88%) of the patients. CNB: core needle biopsy.

cases yielded inadequate material for diagnosis on CNB (n = 3) or a benign diagnosis (n = 1) on CNB. All these 4 cases were malignant on CWC. Additionally, 4 out of the 7 DCIS cases revealed inadequate material after tissue processing for a CNB histological diagnosis (only a

minimal amount of tissue fragments could be recovered from the RPMI fluid), while in 3 of these 4 cases the CWC diagnosis was malignant.
A potential drawback of introducing CWC is that already small and vulnerable tissue specimens become even less suitable for subsequent histological analysis. However, disintegration of vulnerable tissue can already occur during transportation of the material to the pathology laboratory and during preparation for histological analysis anyway. Interestingly, in one of our cases material of two CNBs was submitted for pathological analysis, but one of these specimens was fragmented and lost for further histological analysis, while the other histologically only contained benign breast tissue. In this case the CNB diagnosis was 'benign', but the CWC diagnosis was 'malignant', probably because tumour cells were shed from the fragmented biopsy specimen into the liquid that was used for CWC analysis. As stated before, it is uncertain if such fragments would have yielded a proper diagnosis if they were used for only histological analysis. In a substantial part of these cases, 7 out of 8 cases, a CWC diagnosis was rendered which allowed further management of the patients.
Five out of these 7 cases underwent a second CNB for confirmation of the CWC diagnosis.

Benign lesions
This present study included 51 benign lesions (18 proven by histology on resection specimens and 33 cases with clinical but without histological follow-up). None of the benign cases in our study, however, had the diagnosis suspicious for malignancy or malignant on CWC, while in 9 cases the diagnosis was atypia on CWC.

Patients planning
In our breast clinic we have the agreement to plan patients with a diagnosis malignant and suspicious for malignancy on CWC. Based on the results obtained in this study and the local agreement we created a flow chart illustrating the role of the CWC diagnosis in the management of patients in a sameday breast clinic (Fig. 2). The high sensitivity and specificity of a 'conclusive' (malignant/suspicious for malignancy and benign) diagnosis on CWC allows for rapid further planning of the vast majority of patients.

Conclusion
We conclude that, combining CNB with CWC analysis for the initial diagnostic work-up of breast lesions has some clear advantages: 1. The modified CWC technique can provide a quick and reliable diagnosis of (especially malignant) breast lesions, which is valuable for same-day patient counselling and management planning; 2. Combining CWC with CNB findings can lead to an increase in the number of adequate preoperative diagnoses of malignancy in breast lesions.

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Chapter 8

Contribution of CSF cytology to the management of breast cancer patients with neurological symptoms; a retrospective analysis over two decades

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Submitted

ABSTRACT

Background

To elucidate the contribution of cerebrospinal fluid (CSF) cytology to the management in breast cancer patients who present with neurological symptoms suspected for central nervous system (CNS) metastases.

Methods

From 1989-2009 eighty one patients with breast cancer underwent CSF cytological examination. Relevant tumour characteristics, clinical presentation and radiological findings were scored. The CSF cytological diagnosis was classified according to the 1996 NCI-sponsored conference approach as malignant, suspicious for malignancy, atypical, benign or inadequate.

Results

In 20 years, 145 CSF cytological examinations were performed. Relatively frequent neurological symptoms leading to CSF examination were headache (n = 25), nausea and vomiting (n=19), sensory disturbances (n=16), and cranial nerve dysfunction (n=16), of these headache and nausea/vomiting were most frequently associated with malignant cells in CSF (CSF^+) (in 48% and 53% of the cases, respectively). All 4 patients with the combination of headache and confusion/alteration of mentality had CSF^+. In 10 patients without radiological evidence for CNS metastasis CSF^+ was found. In our series, the value of repeated CSF analysis was very limited, and CSF^+ was strongly correlated with short survival.

Conclusion

A substantial number of patients with neurological symptoms but without radiological abnormalities can have CSF^+. In our series the additional value of repeated cytological examination of CSF was very limited. Our study underscores that CSF cytology is a valuable tool for unequivocal diagnosis of metastatic spread of breast cancer to the CNS, and CSF^+ is a strong predictor for poor survival.

INTRODUCTION

Breast cancer is (after lung cancer) the second most common source of central nervous system (CNS) metastases. Such metastases may present as intraparenchymal, leptomeningeal or dural lesions and as a combination of these. The reported incidence of CNS metastases of breast cancer is 3% to 15%.[1-8] However, because of underdiagnosis and inaccurate reporting, the real incidence of metastatic breast cancer spread to the CNS is likely to be much higher, and an increase in reported incidence can be expected by improving diagnostic tools. Additionally, more effective treatment of primary breast cancer and of systemic (non-CNS) metastases of breast cancer will result in an increase in number of patients that survive long enough to present with CNS metastases.[1-4, 8-11] Metastasis can reach the CNS via the haematogenous route and by direct infiltration from contiguous tumour deposits (e.g. bone metastases) or along the perineural or perivascular spaces. The interval between the diagnosis of primary breast cancer and the manifestation of CNS metastasis including leptomeningeal carcinomatosis (LC; synonym: meningitis carcinomatosa) varies significantly and is related to tumour characteristics such as tumour type and grade.[11-14] Only very rarely CNS metastasis is the first manifestation of breast cancer.[1, 15, 16]

The neurological signs and symptoms of CNS metastases are widely variable and generally caused by increased intracranial pressure or neurological dysfunction due to local effect of the tumour. The neuroradiological findings are very divergent as well. With MRI- and CT-scan mass lesions or density differences can be detected in the brain parenchyma and the spinal cord. Leptomeningeal enhancement can be local or diffuse and can include nodules and enlargement or enhanced cranial nerves. In a substantial number of patients diffuse, 'non-tumefactive' leptomeningeal tumour growth occurs, this form is known as LC and can be very difficult to identify by radiological investigations.

Meanwhile, proof of metastatic disease in/around the CNS is important for rational therapeutic decision making (e.g. systemic and/or intrathecal chemotherapy, radiotherapy, wait and see policy). Cytology of cerebrospinal fluid (CSF) obtained by a lumbar puncture is generally considered as a quick, relatively easy, minimally invasive and inexpensive method to prove the presence or absence of metastatic spread to the CNS, and the diagnostic yield may be increased by analysis of repeated samples.[2, 17] Recent studies on the diagnostic yield of CSF cytology in breast cancer patients are however sparse, and older studies were often based on post mortem investigations and/or data collected in patients with a wide spectrum of different tumors.[18-20]

In order to assess the value of a CSF cytological diagnosis in breast cancer patients presenting with neurological signs and symptoms suspected for metastatic spread to the CNS we conducted a retrospective study in which we compared the results of this cytological analysis with the clinical, neuroradiological, and survival data of these patients.

MATERIALS AND METHODS

Patient population

In a period of 20 years (October 1989-October 2009), 1,106 CSF samples were cytologically analyzed in our department. Of these, 145 samples were obtained from 81 patients known with breast cancer. The patients of this retrospective study were identified through the Dutch nationwide histopathology and cytopathology data network and archive (PALGA).[21] The patients were all female, the age at time of the CSF examination ranged from 33 to 85 years (mean 58.2 years). At time of the CSF analysis 80 patients were known with a breast carcinoma, while in one patient LC was the first presentation of a lobular breast carcinoma. In our series only 6 patients subsequently received chemotherapy and 2 of these patients received also radiotherapy on the brain. This group of patients was not separated in the calculations of survival.

Study design

145 CSF samples of the 81 patients with breast cancer were examined. The samples were obtained with a lumbar puncture by the radiologist or neurologist, using a 21-gauge needle (Bard Peripheral Vascular, Inc., Tempe, Arizona USA). All samples were received fresh, processed in the same laboratory and prepared by cytospin preparation technique. The slides were May Grünwald Giemsa and Papanicolaou stained. In a few cases (n=8) subsequent immunocytochemical analysis was performed, the results of this latter analysis are not included in this study.

According to international guidelines for fine needle aspiration (FNA) cytology, the CSF findings were classified in the categories proposed by the 1996 NCI-sponsored conference approach: malignant, suspicious for malignancy, atypical, benign and inadequate (the latter in this study esp. representing cases with ample admixture of blood). In case of more than one CSF examination, the 'most malignant' result (malignant > suspicious > atypical > benign) was used for the calculations. In this paper, malignant cells in CSF is abbreviated as CSF^+ and benign CSF as CSF^-. The slides were not reviewed.

Vital status and date of death were collected until March 18th 2010 and were obtained from the medical records. The overall (all-cause) survival was calculated from the day of the CSF examination until date of death or end of the follow up (March 18th 2010), whichever came first. The follow up period was at least 180 days long. The quality of life was not analyzed. Details of clinical signs and symptoms were retrieved from the clinical reports. The results of the computerized tomography (CT) scans and (contrast-enhanced) magnetic resonance imaging (MRI) scans of the CNS were retrieved from the radiological files. Metastases on radiology is abbreviated as $Radiol^+$, no metastases on radiology as $Radiol^-$. Metastases in the spine and/or skull may lead to neurological signs and symptoms, therefore breast cancer patients presenting with CNS symptoms due to such osseous metastases are included in the present study as well. The CSF cytological diagnoses were compared with the clinical, radiological, histopathological and survival data.

Statistical analysis

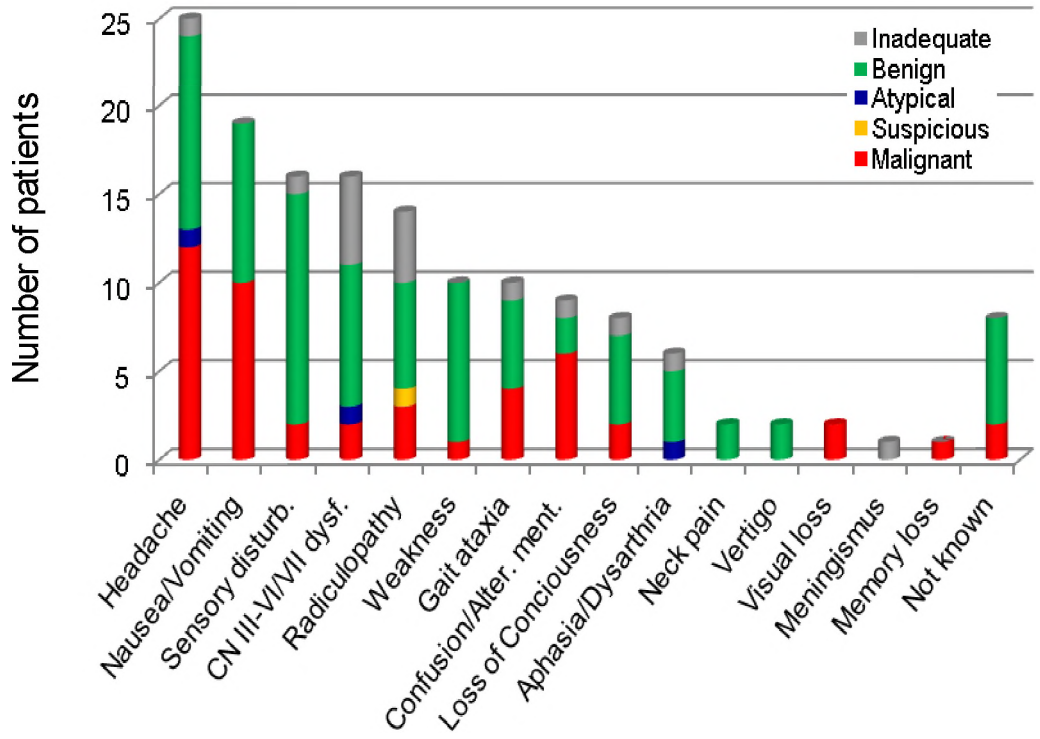
Survival analysis was performed using the Kaplan-Meier method with the log rank test to test survival differences between CSF cytology diagnosis and radiology diagnosis groups. Values of $p<.05$ were considered significant. The statistical software package SPSS 15.0 (SPSS Inc., Chicago, Illinois) was used for analysis.

RESULTS

Neurological signs and symptoms

In the clinical records of 73 patients, 15 different neurological signs or symptoms were described that elicited further radiologic and pathologic evaluation. In 8 of the 81 patients the exact nature of the neurological signs or symptoms could not be retrieved. Many patients showed two (n=20) or more (n=25) signs or symptoms simultaneously. For this study a

Figure 1 Neurological signs and symptoms (in decreasing number) of the 81 patients related to the results of the CSF cytological examination



maximum of three was scored per patient. The most frequently recorded symptoms were headache (n=25), nausea/vomiting (n=19), sensory disturbances (n=16), and cranial nerve III, IV, V VI and/or VII dysfunction (n=16) (Fig. 1). CSF^{+} was found in respectively 48%, 53%, 13% and 13% of the patients with these symptoms. In 62% of the 13 patients presenting with the combination of headache and nausea/vomiting and in all 4 patients with the combination of headache and confusion/alteration of mentality malignant cells were found in the CSF.

CSF cytology and survival

In Figure 2, CSF outcome is presented for each type of breast cancer. Malignant cells in CSF were relatively frequently found in patients with ductal carcinoma grade 3 (n=17,4%) and in lobular carcinoma (n=5,38%). In the remaining tumour categories only one out of 25 patients showed malignant cells in the CSF (n=1,4%).

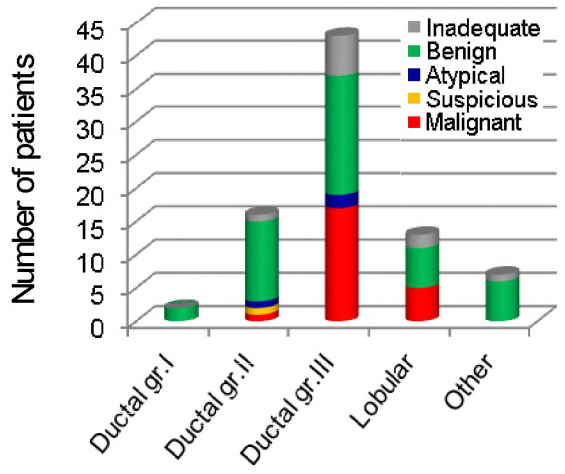


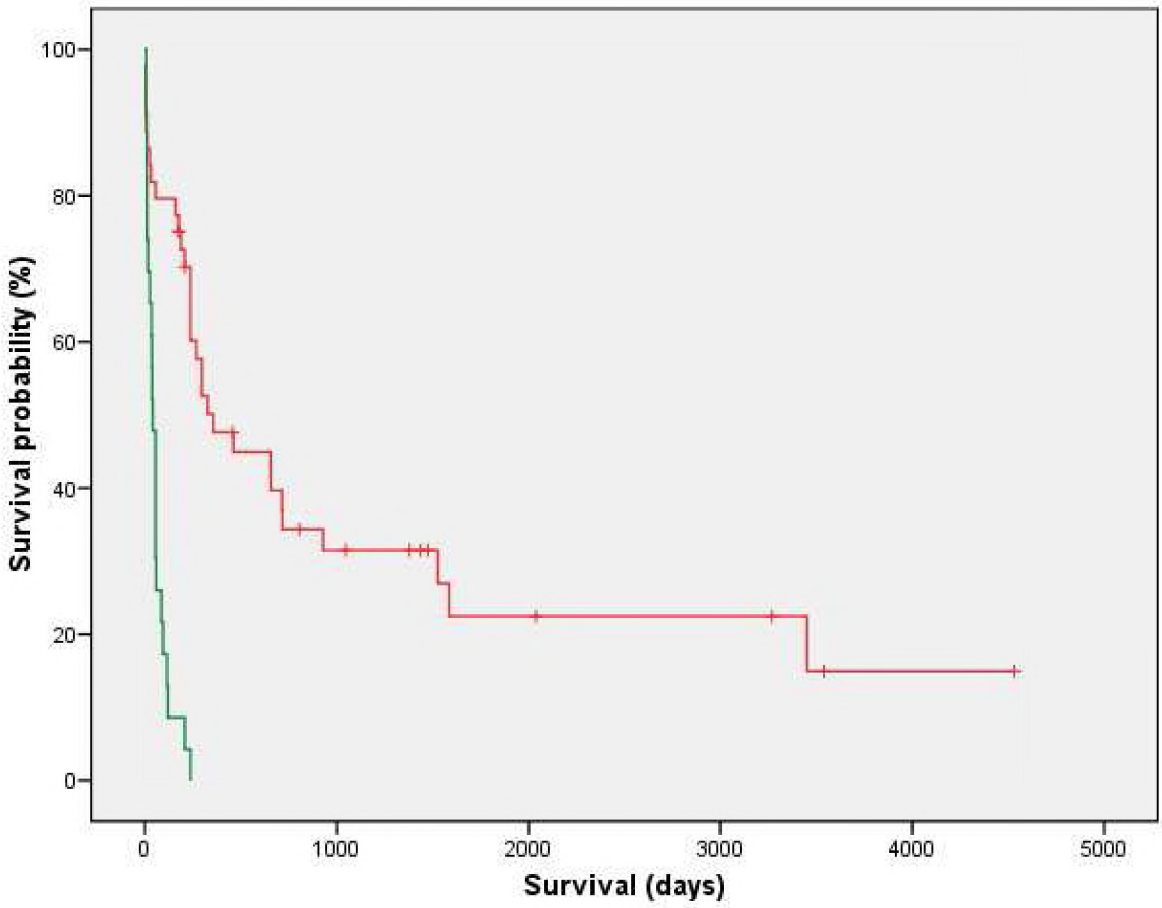
Figure 2 Tumour type primary breast carcinoma (ductal carcinoma grade I, II and III; lobular carcinoma, and other carcinomas (mucinous, apocrine, tubular, combined tubular/lobular, metaplastic carcinoma and ductal carcinoma in situ grade II)) related to cytological diagnosis of the CSF.

The patients included in this study underwent one (n=54), 2 (n=16), 3 (n=6), 4 (n=1), 6 (n=1), 8 (n=1), 10 (n=1) or 13 (n=1) CSF examinations. In one patient with repeated CSF analysis, the diagnosis changed from suspicious for malignancy into malignant, in another patient the diagnosis changed from benign to atypical. Multiple (> 6) CSF cytological examinations were performed in patients to monitor the effect of chemo- and/or radiotherapy.

The survival functions for patients with CSF^+ or CSF^- are shown in figure 3. The follow up varied from 3 to 4530 days and was at least 180 days in patients still alive in March 2010. In the group of 23 patients with the CSF^+ all patients died within 11-240 days (median 45 days). Of the 44 patients with CSF^-, 31 died within 3-3450 days (median survival of CSF^- patients 360 days, log rank test $p<0.001$). Eleven of these 31 patients with CSF^- died within 6 months, 4 of these 11 patients showed LC and/or mass lesion, 2 osseous metastases in skull and/or spine on radiology, while 5 patients showed no radiological abnormalities (n=3) or had no radiology (n=2). However, 6 of these 11 patients suffered from systemic metastases and 7 had hypercalcaemia suggesting osseous metastases. Thirteen of the 44 CSF^- patients were still alive at the end of the study, follow up ranging from 180-4530 days.

The one patient with the CSF cytological diagnosis suspicious for carcinoma died 1080 days after CSF diagnosis, while the 3 patients with a CSF diagnosis atypical were still alive at the end of the study, the follow up ranging from 420-860 days. Seven of the 10 patients with a CSF cytological diagnosis 'inadequate' died in 14 to 1442 days (mean 311 days), the remaining three patients were still alive 1080 to 1578 days after CSF examination.

Figure 3 Association between the CFS results and survival of total 67 patients. (Kaplan Meier Curves) Median survival: 45 days (CSF^+) and 360 days (CSF^-).
CSF^+, malignant cells in CSF; CSF^-, no malignant cells in CSF.



Number of patients at risk						
Days	0	1000	2000	3000	4000	5000
CSF^-	44	11	5	4	1	0
CSF^+	23	0	0	0	0	0

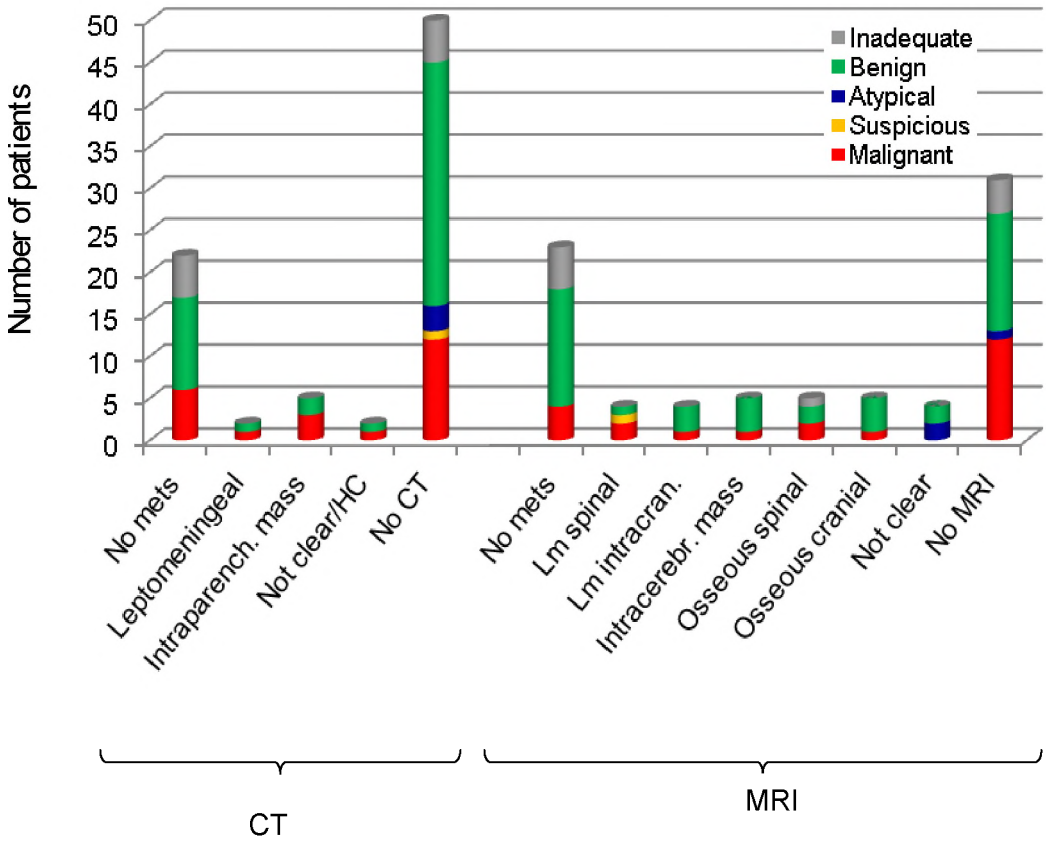
CSF cytology and survival after treatment

Of the 6 patients who received chemotherapy (in 2 patients combined with radiotherapy on the brain) five were CSF^+ and one CSF^-. The outcomes of this small group are incorporated in the overall calculations. However, the survival in this group of 6 patients ranged from 41-240 days (mean 122 days) after CSF examination. The CSF^- patient in this group had osseous metastases in the skull and died 60 days after CSF examination.

Radiologic findings and survival

Of the 81 patients, 31 had contrast enhanced CT scan (esp. in the first decade of the study period from which patients were retrieved) and 50 contrast enhanced MRI scan (esp. in the second half of the study period). Twelve of these patients had both CT and MRI examination of the CNS at the time of presentation with neurological signs and/or symptoms.

Figure 4 The results of the CT- and MRI scan related with the results of the CSF examinations of the 81 patients. Mets, metastases; not clear, mass lesion or leptomeningeal metastases; HC, hydrocephalus; LM, leptomeningeal.

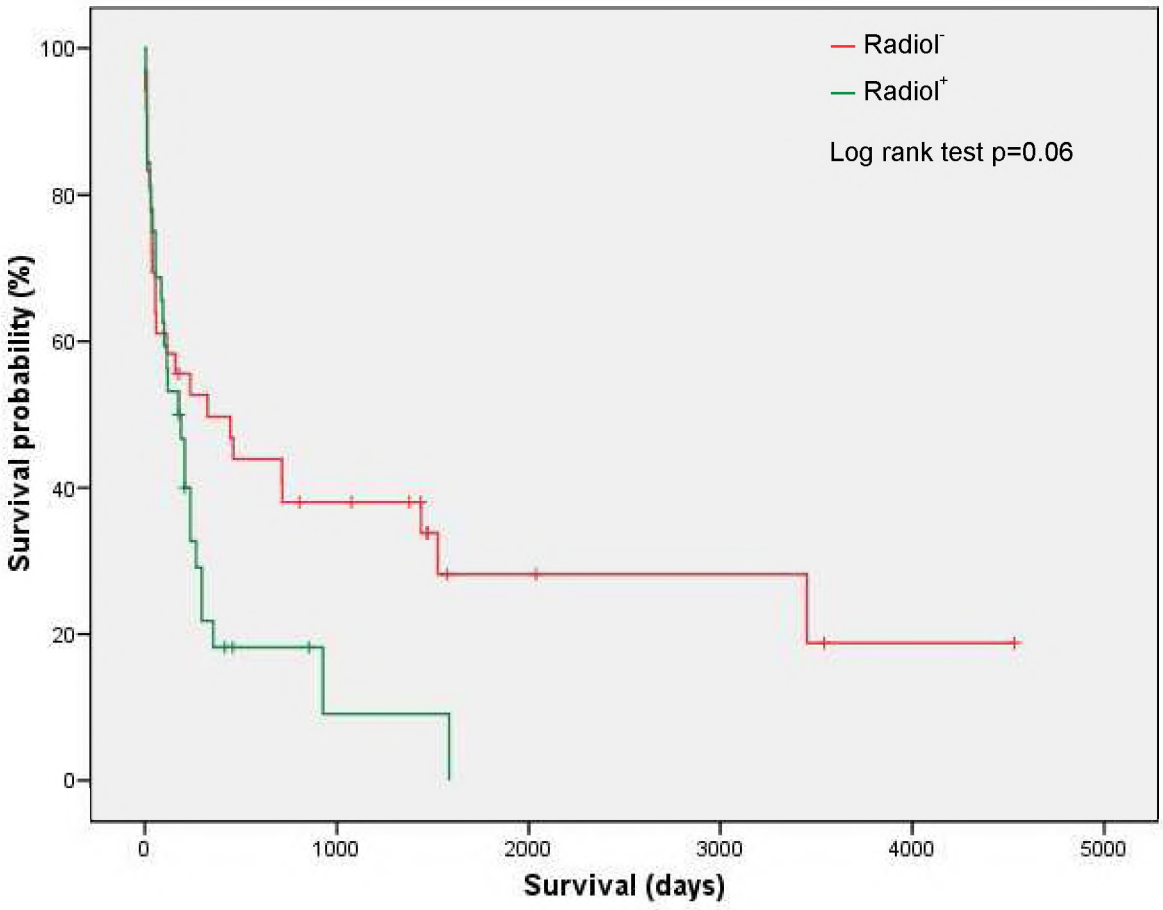


CT-scan suggested: no metastasis (n=22/31), LC (n=2/31), intracerebral mass (n=5/31), hydrocephalus (n=1/31) and in one case it was hard to differentiate between a mass lesion or LC. MRI-scan revealed: no metastasis (23/50), spinal (n=4/50) and/or cranial LC (n=4/50), intracerebral mass lesions (n=5/50) and in 4 cases it was impossible to differentiate between a mass lesion or LC. One out of four cranial LC cases had also an intracrerebral mass lesion. Osseous metastases were also detected on MRI (n=10/50), in one of these patients combined with LC. The CSF outcomes are related with the radiologic findings in Figure 4. Of note, in 6 patients with CT scans and 4 patients with MRI scans no explanation was found for the neurological symptoms, however CSF examination in these patients did reveal tumour cells.

Twelve of the 81 patients underwent both contrast enhanced CT- and MRI scan. In 9 patients the findings on CT and MRI were consistent. In 3 patients MRI investigation revealed metastasis and CT imaging did not. Additionally, in 4 patients, radiological examination revealed disc herniation (n=2) and cerebral infarction (n=2) instead of malignancy as a cause of the neurological symptoms.

Twelve of the 81 patients had no CT or MRI scan. In this group of 12 patients CSF was malignant, atypical and benign in 3, 1 and 8 patients, respectively. Nine of these patients died within 6 to 660 days.

Figure 5 Association between the CT and/or MRI findings. (Kaplan Meier Curves) Median survival: 180 days (Radiol$^+$) and 330 days (Radiol$^-$).
CSF$^+$, malignant cells in CSF; CSF$^-$, no malignant cells in CSF. Radiol$^+$, malignancy on radiology; Radiol$^-$, no malignancy on radiology.

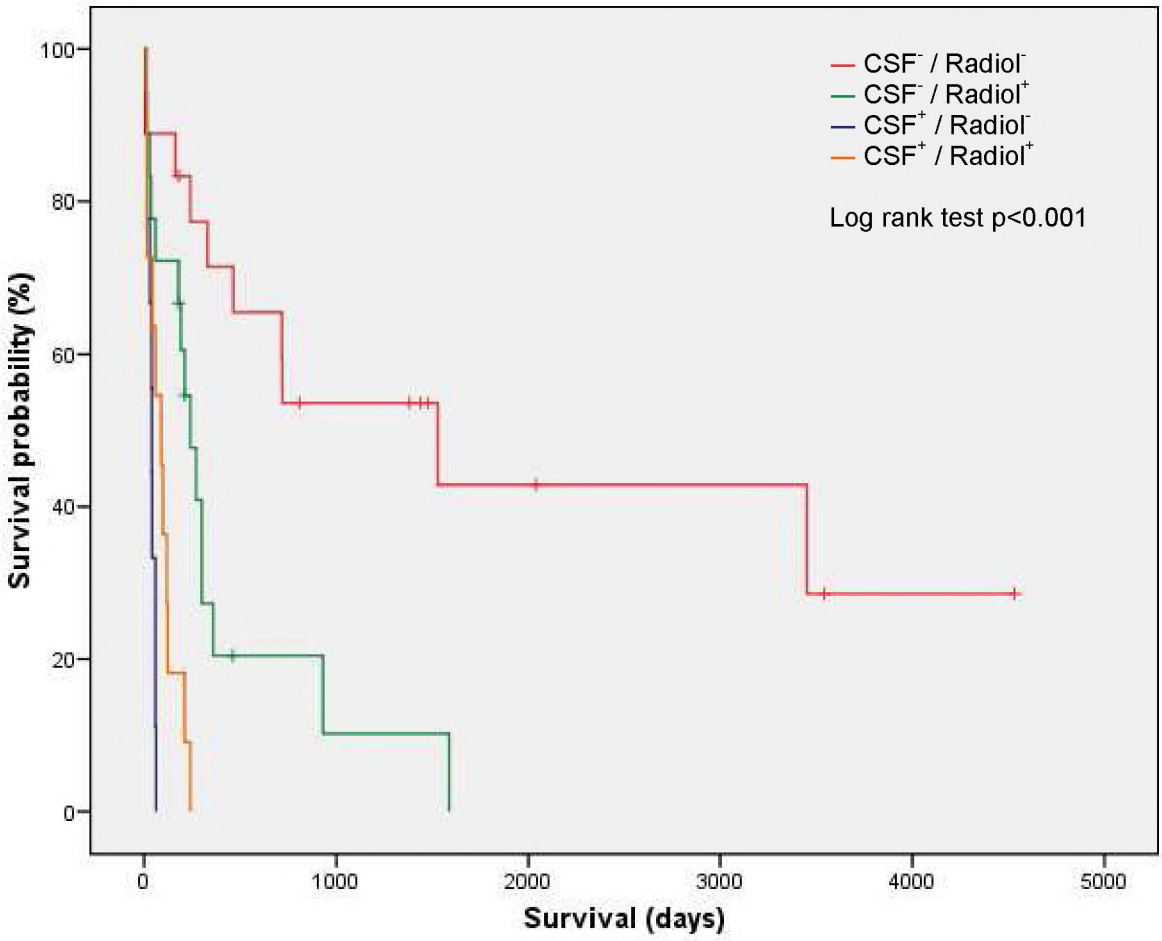


Number of patients at risk

Days	0	1000	2000	3000	4000	5000
Radiol$^-$	36	12	4	3	1	0
Radiol$^+$	32	1	0	0	0	0

We also examined the association between radiologic findings and survival. (figure 5) Metastases on CT and/or MRI were seen in 32 patients. Of them, 27 patients died in 9-1590 days (median survival 180 days). Five patients were still alive with a follow up of 180-860 days (1 with an intradural metastasis in the spine, 3 with LC and/or a mass lesion in the brain,

Figure 6 Association between the combined CT/ MRI and CSF results and survival. (Kaplan Meier Curves) Median survival: 90 days (CSF^+ , $Radiol^+$), 41 days (CSF^+ , $Radiol^-$), 240 days (CSF^- , $Radiol^+$) and 1530 days (CSF^-, $Radiol^-$).
CSF^+, malignant cells in CSF; CSF^-, no malignant cells in CSF. $Radiol^+$, malignancy on radiology; $Radiol^-$, no malignancy on radiology.



Number of patients at risk						
Days	0	1000	2000	3000	4000	5000
CSF^- / $Radiol^-$	18	8	4	3	1	0
CSF^- / $Radiol^+$	18	1	0	0	0	0
CSF^+ / $Radiol^-$	9	0	0	0	0	0
CSF^+ / $Radiol^+$	11	0	0	0	0	0

and 1 with an osseous metastasis in the skull). $Radiol^-$ was seen in 36 patients, with a follow up of 3-4530 days, median survival 330 days. Twenty five of them died within 5-3450 days. Of the 17 patients that died relatively rapidly (within 6 months), 9 had CSF^+, 5 had systemic disease and 12 patients had osseous metastases in the body (hypercalcaemia). Survival was better in $Radiol^-$ than in $Radiol^+$ patients, although not statistically significant (log rank test

p=0.06).
Finally, we compared the combined results of CSF cytology and radiological findings with survival. (Fig 6) The median survival for patients with $CSF^+/Radiol^+$, $CSF^+/Radiol^-$, $CSF^-/Radiol^+$ and $CSF^-/Radiol^-$ was 90, 41, 240 and 1530 days, respectively. Retrospective analysis of the cases causing the steep part of the $CSF^-/Radiol^-$ curve showed that these patients suffered from systemic disease and/or hypercalcaemia. The flat part of the $CSF^-/Radiol^+$ slope was caused by 20 patients with a survival of up to 1590 days. Six of these $Radiol^+$ patients had skull and/or spine metastases rather than metastases in the intradural compartment.

DISCUSSION

In the present study we evaluated the contribution of cytological examination of the CSF using Papanicolaou and May Grunwald Giemsa stains in breast cancer patients presenting with neurological signs and symptoms in a period of two decades. One of the first reports on carcinoma cells in the CSF is from Dufour in 1904.[22] Interestingly, many of the frequently cited results in literature on this topic are relatively old and/or based on autopsy reports. [3, 6, 23]
Due to improved therapeutic efficacy in patients with systemic metastases of (breast) cancer, the frequency of CNS metastasis has substantially increased.[1] The reported incidence of CNS metastases of breast tumours in clinical series is 3-15%, thereby it represents the most frequently detected CNS metastasis in large studies.[2, 8, 15 11, 14] It is likely that the real incidence is even much higher as autopsy studies reported incidences ranging from 18% to 30%.[9, 18-20] Metastatic carcinoma cells reach the leptomeninges and subarachnoidal space by haematogenous spread or by direct extension from solid tumour lesions and subsequent dissemination in the CSF compartment. Examination of the CSF is theoretically a good modality for confirmation of the existence of such metastatic spread to the CNS.
CNS metastasis can generate multiple neurological signs and/or symptoms.[1-3, 7, 24] The 4 most frequent symptoms in our series were headache, nausea/vomiting, sensory disturbance (extremities and cauda equina region), and cranial nerve dysfunction. Of note, no single neurological sign or symptom, nor a particular combination of these symptoms was a strong predictor of finding malignant cells in the CSF (except for the combination of headache and confusion/altered mentality, but this latter group consisted of only 4 patients). Furthermore, of course neurological symptoms in breast cancer patients can also very well be due to non-neoplastic disease. This is illustrated by the fact that in our series disc herniation and cerebral infarction was found in 4 patients as an explanation for their neurological symptoms and, indirectly, by the fact that a substantial number of the patients in our series (especially those with CSF^- and $Radiol^-$) showed a much longer survival than would be expected when CNS metastasis was indeed present.
Ductal and lobular carcinoma are the most frequent types of breast carcinomas. As expected, this was also true in our study (91% of total number of breast carcinomas). Moreover, of all diagnoses 'malignant'on CSF cytology 74% were metastatic ductal carcinomas grade III. LC is usually seen in patients with systemic disease, or can be seen after a disease-free period and occasionally, in the absence of a primary tumour.[1, 11, 15, 16] In our study, in one patient the

initial manifestation of a lobular breast carcinoma was LC with malignant cells in the CSF.
Twenty seven patients (33%) underwent more than one CSF examination. While it is stated in the literature that multiple punctures increase the diagnostic accuracy of CSF cytology, in our series repeated punctures only led to a slight difference in diagnosis in two cases (suspicious for carcinoma became malignant in one case and benign became to be atypical in another).[1-3, 23, 25] In this series repeated cytological analyses thus did not really help to improve the CSF cytology diagnosis.
In the literature the mean survival of untreated metastases to the central nervous system of breast carcinomas is given as 4 to 6 weeks, the mean overall survival of patients treated for LC is given as 6 to 7.5 months, while survival can be up to 16 months or incidentally even more than 30 months if surgical excision of a solitary metastasis is performed. [4, 10, 11, 14, 26, 27]
Our study showed a significant difference in survival between patients with a benign (CSF^-) versus malignant (CSF^+) diagnosis on CSF examination. The 23 CSF^+ patients showed a median survival of 45 days, while 44 CSF^- patients had a median survival of 360 days. Of this latter group, 11 patients died within 6 months, of these 4 patients showed on radiology LC and/or mass lesions and 2 patients revealed osseous metastases.
Comparison of the CT- and MRI scan results with survival indicates that, if no metastases are seen the survival is better than when metastastic disease can be demonstrated and vice versa, although this was not statistically significant. Patients with metastases on radiology had a median survival of 180 days, while patients with CSF^+ showed a median survival of 45 days. CSF^+ thus predicts a somewhat worse survival for the patient than metastases on radiology. The discrepancy between this difference on survival between radiological and CSF findings may partly be explained by the fact that intraosseous but extradural metastatic lesions on radiology may have less effect on survival than 'real' CNS metastasis with spread of tumour cells into the CSF.
It is important to realize that the group of patients presented in this study represents a selection of the whole group of breast carcinoma patients with CNS metastases as not in every breast cancer patient that develops neurological symptoms CSF examination will be performed. Moreover, in the nineties of the previous century CSF examination was in our centre generally the first diagnostic modality applied, followed by CT-scan examination, while the current national guidelines in the Netherlands for patients suspected for CNS metastasis of (breast) cancer advise to first perform a MRI scan, and only if no metastases are seen on radiology CSF examination is advocated. This change means that it would be less easy nowadays to collect a fair number of patients that had both metastatic disease on radiological and CSF cytological examination. Also, it is generally accepted that MRI scans are superior to CT scans when it comes to radiological detection of (metastatic) diseases of the CNS.[28, 29]
These changes in indications to perform different diagnostic tests and improved techniques will thus have an influence on figures as presented here, and further improvements in diagnostic techniques are almost continuously made. For instance, recently a nomogram was published that can be used to calculate the risk on CNS metastasis for patients with breast cancer. Based on such a nomogram high-risk patients can be identified and selected for e.g. upfront neo-adjuvant therapy in order to prevent the occurrence of CNS metastases.[11] Still, as soon as breast cancer patients present with neurological problems additional investigations are

warranted. Of course, radiological detection is now much better than 20 years ago, and detection of metastatic disease in/around the CNS was probably more recently further improved with the introduction of 3Tesla MR scanners.[28, 30] Also, morphological analysis of the CSF is now often supplemented by immunocytochemical staining, and in addition molecular analysis is increasingly used in diagnostic pathology to improve the detection of malignancy in the CSF.[31-36]

Although our present, retrospective study thus has its limitations, the results do allow us to draw some meaningful conclusions: 1.CSF cytology leads to an unequivocal diagnosis of metastatic spread to the CNS in a substantial number of breast cancer patients; 2. Malignant cells in the CSF is a strong predictor for poor survival of these patients; 3. Combination of both diagnostic modalities (radiology and CSF examination) can have extra value; 4. The additional value of repeated cytological examination of CSF was in our series very limited; 5. Malignant CSF cytology was relatively frequently found in grade III ductal carcinoma patients and in lobular carcinoma patients; 6. Single neurological signs or symptoms nor combinations there-of are a good predictor for the chance of finding malignant cells in the CSF.

Conclusion

We conclude that cytological examination of the CSF in breast cancer patients presenting with neurological symptoms is still a valuable, inexpensive, minimally invasive tool for unequival demonstration of CNS metastasis of breast cancer. Esp. when malignant cells are demonstrated in the CSF, this tool provides important prognostic information that will aid in therapeutic decision making. However, in order to further improve evidence-based clinical practice in this area, larger studies are needed to assess the exact value of the improved radiological and pathological diagnostic approaches in patients suspected for CNS metastases of breast cancer.

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Chapter 9

Summary and conclusions

The studies presented in this thesis focus on the value of cytological examination in the diagnostic work-up and management of patients with breast lesions, especially for a 'same-day breast clinic'. In such a clinic on the day of the first outpatient visit the patient is informed of the nature of the breast lesion (benign or malignant) and further treatment is planned. In addition, in this thesis the value of cytological examination of the cerebrospinal fluid in breast cancer patients presenting with neurological symptoms was studied.

In **chapter 2** we reported our diagnostic experience with a monolayer preparation (MP) technique of breast FNA, employing the Hettich centrifuge. The FNA diagnoses were classified into one of the 5 diagnostic categories as proposed by the 1996 National Cancer Institute-sponsored conference approach: malignant (C5), suspicious for malignancy (C4), atypical (C3), benign (C2) and inadequate (C1). The reference standard was the histological follow-up. A conclusive FNA diagnosis was defined as C2 (in lesions benign in follow-up) and C5 (in lesions malignant on histology). In this retrospective study aspirates processed by conventional smears (CS) (1992-1996) were compared with aspirates processed by MP (1999-2003). We demonstrated that MP-prepared FNA significantly more often resulted in a conclusive diagnosis compared with CS-prepared FNA.

In **chapter 3** repeat FNA and CNB were compared with regard to their ability to provide a clinically useful diagnosis after indeterminate primary breast FNA. After correction for patient age, mammographic presentation, clinical findings, tumour size, freehand or image guided aspiration, the study reveals significantly better results with the CNB than with the repeat FNA.

The value of cytological examination of nipple discharge (ND) in clinical decision-making is reported in **chapter 4.** We found a very low additional diagnostic value compared to clinical evaluation and subsequent histological examination in both benign and malignant breast lesions that cause pathologic ND. The sensitivity of ND cytology is low, but a positive finding has acceptable specificity and is therefore alarming. Interestingly, ND colour (esp. 'bloody' vs. 'milky') examination had a higher sensitivity and only a slightly lower specificity when compared with ND cytology. The specificity of the cytological examination of bloody ND was significantly lower than in otherwise coloured ND. Our results indicate that cytological examination of ND has limited value for clinical decision making If a carcinoma is not evident in ND cytology, the clinician must be aware of the low relevance and therefore possibly misleading results of the ND.

In **chapter 5** the value of FNA in the work-up of male breast lesions was determined over a period of 15 years. A substantial number of inadequate FNAs were obtained in this patient group which can be explained by the fact that gynaecomastia is the predominant nature of male breast lesions. However, due to the good cytologic correlations in the group of malignant lesions, cytology still can be considered as an important diagnostic tool in the work-up of clinically suspect male breast lesions. The results of our study were comparable to those in studies reported in the recent literature.

In **chapter 6** a preclinical study is described investigating a modified method of TI and CW cytology in patients with breast cancer. (ref) Fresh breast specimen, obtained after surgery, were biopsied by a core needle in a laboratory setting. With the modified technique TI and CW slides were made and categorized as inadequate (C1), benign (C2), atypical (C3), suspicious for malignancy (C4) and malignant (C5) and compared with the histologic CNB results (ref). The study showed only a slight difference between CW and TI cytology. However, because of the better morphology and the advantages of the monolayer technique that was applied in preparation of CW specimen, the CW cytology technique was preferred and subsequently introduced into the clinical setting.

The first clinical data on CW cytological diagnosis in this context are presented in **chapter 7**. CW cytology and the CNB histological diagnoses obtained from 226 breast lesions were correlated with the histopathology of subsequently obtained resection specimens. CW cytology resulted in good sensitivity and specificity compared to the histological resection diagnosis. Thus, the introduced modified CW cytology technique can provide a reliable preliminary indication of the likely CNB diagnosis and thereby a valuable tool suitably for same-day patient counselling and planning of further management.

In order to evaluate the value of cytological examination of the CSF in patients known with breast cancer that developed neurological signs and/or symptoms we designed a retrospective study. (**chapter 8**). We revealed that in a substantial number of patients with neurological symptoms but without radiological abnormalities, malignant cells can be found in the CSF. In contradiction with the literature the additional value of repeated cytological examination of CSF was very limited. The study underscored that CSF cytology is a valuable tool for unequivocal diagnosis of metastatic spread of breast cancer to the CNS. Also, the detection of malignant cells in the CSF proved to be a strong predictor for poor survival.

We are well aware of the controversy concerning the role of cytological examination in the management of breast abnormalities in general and non palpable breast lesion in particular. Based on the studies described in this thesis we conclude that cytological examination provides a rapid and reliable diagnosis in many patients and is therefore still a valuable aid in the diagnostic work-up of patients with breast lesions, esp. in a 'same-day breast clinic' in which ultra-rapid processing of biopsies for histological analysis is not (always) available. Other advantages of cytological analysis are that it is relatively inexpensive and technically easy to perform. However, for optimal preparation and evaluation of cytological specimens of breast lesions skilled personnel is crucial, and the diagnosis made on cytological specimens is usually less specific than diagnoses based on biopsy material (e.g. 'malignant' or 'carcinoma' on cytology vs. type of cancer and malignancy grade on histological analysis). Furthermore, using only the cytological approach leaves little material for additional immunocytochemical and/or molecular investigations. An important finding reported in this thesis is that rapid cytological analysis can be combined with histological examination of core needle biopsy material of a breast lesion without compromising the quality of the histological diagnosis, thereby significantly facilitating patient management in a 'same-day breast clinic'.

We conclude that cytological examination is still a very valuable tool in the diagnostic work-up and management of patients with breast lesions/breast cancer, and that it is important to maintain the high level of diagnostic skills in this field.

Samenvatting en conclusies

De studies in dit proefschrift richten zich op de waarde van cytologisch onderzoek in de preoperatieve diagnostiek en het plannen van het verdere onderzoek en de behandeling van patiënten met borstafwijkingen, vooral belangrijk voor een 'zelfde-dag-borstkliniek' ('same-day breast clinic'). In een dergelijk centrum wordt op de dag van het eerste poliklinische bezoek de patiënt geïnformeerd over de aard van de borstlaesie (goedaardig of kwaadaardig) alsmede het verdere vervolgonderzoek en behandeling gepland. Tevens is in het kader van dit proefschrift de waarde van cytologisch onderzoek van de hersenvloeistof (liquor) onderzocht bij patiënten bekend met borstkanker die zich presenteren met neurologische klachten.

In **hoofdstuk 2** rapporteren we onze diagnostische ervaring met de cytologische 'dunnelaag' techniek van dunne naald puncties (Fine Needle Aspiration: FNA) van borstafwijkingen, gebruik makend van de Hettich centrifuge. De FNA diagnosen worden ingedeeld in een van de 5 diagnostische categorieën, zoals beschreven in de 'Consensus statement on FNA of the breast' geformuleerd en geaccordeerd tijdens de in 1996 door het National Cancer Institute gesponsorde conferentie: maligne/kwaadaardig (C5), verdacht voor maligniteit (C4), atypie (C3), benigne/goedaardig (C2) en onvoldoende diagnostische cellen/materiaal (C1). De referentie-standaard was de diagnose op het weefsel (resectie preparaat) dat in de vervolgbehandeling door de chirurg werd weggenomen (de histologische follow-up). Een conclusieve FNA diagnose werd gedefinieerd als C2 (histologische follow-up goedaardig) en C5 (histologische follow-up maligne). In deze retrospectieve studie werden de puncties verwerkt met de 'dunnelaag' techniek (1999-2003) vergeleken met de puncties verwerkt tot conventionele uitstrijkjes (1992-1996). Uit de studie bleek dat de met 'dunnelaag' techniek verwerkte puncties significant vaker resulteerden in een conclusieve diagnose dan de puncties die verwerkt waren tot conventionele uitstrijken.

In **hoofdstuk 3** worden de resultaten van de herhaalde cytologische punctie vergeleken met het holle naald weefsel biopt (Core Needle Biopsy: CNB) welke werden afgenomen nadat de eerste punctie uit een borstafwijking een 'niet-sluitende' (niet informatieve) diagnose had opgeleverd. Na correctie voor leeftijd van de patiënt, het mammografische beeld, klinische bevindingen, tumorgrootte, en het al dan niet echogeleid puncteren, blijkt dat er significant betere resultaten worden behaald met de CNB dan met het herhalen van de FNA.

Over de waarde van cytologisch onderzoek van tepelvocht in de klinische besluitvorming wordt gerapporteerd in **hoofdstuk 4**. We vonden een zeer lage aanvullende diagnostische waarde van het cytologisch onderzoek in vergelijking met de klinische evaluatie en het daaropvolgende weefsel (histologisch) onderzoek in zowel goedaardige als kwaadaardige laesies van borst die pathologisch tepelvocht veroorzaakten. De gevoeligheid (sensitiviteit) van de cytologische beoordeling van tepelvocht is laag, maar het vinden van een maligniteit heeft een aanvaardbare specificiteit en is daarom alarmerend. Bijzonder was de bevinding dat de beoordeling van de kleur van tepelvocht (vooral 'bloedig' versus 'melkachtig') een hogere sensitiviteit en slechts een iets lagere specificiteit had in vergelijking met de cytologische beoordeling van het tepelvocht. De specificiteit van het cytologisch onderzoek van bloedig

tepelvocht was beduidend lager ten opzichte van anders gekleurd tepelvocht. Onze resultaten geven aan dat cytologisch onderzoek van tepelvocht beperkte waarde heeft voor de klinische besluitvorming. Indien bij cytologisch onderzoek niet een aperte maligniteit in het tepelvocht wordt aangetroffen moet de arts zich bewust zijn van de lage sensitiviteit en dus mogelijk misleidende informatie de cytologische beoordeling van het tepelvocht kan opleveren.

In **hoofdstuk 5** wordt de waarde van de FNA in de diagnostiek van de borstafwijkingen bij de man beschreven in een retrospectieve studie over een periode van 15 jaar. Een aanzienlijk aantal 'niet-sluitende' diagnosen op de FNA werd afgegeven in deze groep patiënten, hetgeen verklaard kan worden door het feit dat het veelal een gynaecomastie betrof. Dit is cytologisch, gezien de aard van de laesie, lastig te diagnostiseren. Echter, vanwege de goede cytologische correlaties in de groep van maligne laesies kan de FNA nog steeds worden beschouwd als een belangrijk diagnostisch hulpmiddel in de diagnostiek van klinisch verdachte laesies in de borst van de man. De resultaten van onze studie waren vergelijkbaar met de gerapporteerde resultaten van studies uit de recente literatuur.

In **hoofdstuk 6** worden in een preklinische studie twee gemodificeerde cytologische technieken beschreven waarbij celmateriaal van patiënten met borstkanker verkregen wordt van een dikke naald weefselbiopt door middel van het wassen van het biopt (Core Wash: CW) danwel het biopt tegen een glaasje aan te drukken (Touch Imprint: TI). In het laboratorium ontvangen, niet-formaldehyde gefixeerde borstweefselresectie preparaten met haardvormige afwijkingen werden gebiopteerd met een 18-gauge holle naald, en van het biopsie materiaal werden TI en CW glaasjes voor cytologische beoordelingen gemaakt. De diagnosen werden gecategoriseerd als: onvoldoende diagnostische cellen/materiaal (C1), benigne (C2), atypie (C3), verdacht voor maligniteit (C4) of maligne (C5) en vergeleken met de histologische resultaten van de CNB. De studie toonde slechts een klein verschil aan in de resultaten tussen CW en TI cytologie. Echter, vanwege de betere morfologie en de voordelen van de 'dunnelaag' techniek werd gekozen voor de CW techniek, welke vervolgens werd ingevoerd in de klinische praktijk.

De eerste klinische gegevens over de CW diagnostiek worden gepresenteerd in **hoofdstuk 7**. De resultaten van de CW cytologie en de CNB histologie, verkregen van 226 afwijkingen in de borst, werden vergeleken met de histologische resultaten van de later verkregen resectie preparaten. De CW cytologie resulteerde in een goede sensitiviteit en specificiteit in vergelijking met de histologische diagnose van het resectie preparaat. De applicatie van de gemodificeerde CW cytologie techniek kan zorgen voor een betrouwbare indicatie van de verwachte CNB diagnose en daarmee een waardevol instrument zijn bij het plannen van verder vervolgonderzoek en behandeling van patiënten die een 'zelfde-dag-borstkliniek' bezoeken.

Hoofdstuk 8 beschrijft een onderzoek waarbij in een retrospectieve studie, de waarde van het cytologisch onderzoek van het hersenvocht (liquor) bij patiënten bekend met borstkanker en bij wie neurologische klachten werden vastgesteld. Bij een substantieel aantal patiënten met neurologische symptomen, maar zonder radiologische afwijkingen, werden kwaadaardige

cellen in de liquor aangetroffen. In tegenspraak met de literatuur was de toegevoegde waarde van herhaald cytologisch onderzoek van de liquor zeer beperkt. De studie onderstreept dat liquor cytologie een waardevol instrument is voor een eenduidige diagnose van de metastatische verspreiding van borstkanker naar het centraal zenuwstelsel. Het vaststellen van kwaadaardige cellen in de liquor bleek een sterke voorspeller te zijn voor een korte overleving.

Wij zijn ons bewust van de controverse over de rol van cytologisch onderzoek in de diagnostiek van borstafwijkingen in het algemeen en in het bijzonder van niet-palpabele borstafwijkingen. Gebaseerd op de studies beschreven in dit proefschrift kunnen we concluderen dat cytologisch onderzoek een snelle en betrouwbare diagnose biedt bij veel patiënten en daarom nog steeds een waardevolle steun is in de diagnostiek van patiënten met borstafwijkingen, vooral in de 'zelfde-dag-borstkliniek' waarin ultra-snelle verwerking van biopten voor histologische analyse niet (altijd) beschikbaar is. Andere voordelen van de cytologische analyse zijn: het is relatief goedkoop en technisch eenvoudig uit te voeren. Echter, voor een optimale bereiding en evaluatie van de cytologische preparaten van de borstafwijkingen is geschoold personeel van cruciaal belang, verder is de cytologische diagnose veelal minder specifiek dan de histologische diagnose die gesteld wordt op biopsie materiaal (bv. 'carcinoom' op basis van cytologisch onderzoek versus type kanker en maligniteitsgraad op basis van histologische analyse). Bovendien laat een puur cytologische benadering veelal weinig materiaal over voor extra immunocytochemische en/of moleculair onderzoekn. Een belangrijke bevinding in dit proefschrift is dat een snelle cytologische analyse kan worden gecombineerd met het histologisch onderzoek van een dikke naald biopsie van een borstafwijking, zonder dat de kwaliteit van de histologische diagnose wordt aangetast. Daardoor kan diagnostiek en het verder plannen van behandeling van de patiënt in een 'zelfde-dag-borstkliniek', beter worden vormgegeven.
We concluderen derhalve dat cytologisch onderzoek nog steeds een zeer waardevol instrument is in de diagnostiek en behandeling van patiënten met laesies in de borst / borstkanker, en dat het belangrijk is om het hoge niveau van de diagnostische vaardigheden op dit gebied te handhaven.

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Curriculum Vitae

Carla (Carolina Alphonsina Petronella) Wauters is geboren op 29 maart 1957 te Hulst in Zeeuws-Vlaanderen, Zeeland. In Hulst heeft zij het Atheneum B aan de Jansenius Scholengemeenschap gevolgd. In 1978 werd aangevangen met de studie Geneeskunde aan de Rijks Universiteit te Utrecht, deze studie werd afgerond in 1986. Vervolgens was zij tussen 1986 en 1989 werkzaam als assistent-niet-in-opleiding (agnio) op de afdeling Algemene Heelkunde in het St. Antonius Ziekenhuis te Nieuwegein en op de afdelingen KNO en Heelkunde in het Antoni van Leeuwenhoek Ziekenhuis te Amsterdam (op laatstgenoemde afdeling met focus op borstkanker).

In 1989 werd Carla Wauters aangesteld als agnio Pathologie in het Diagnostisch Centrum SSDZ te Delft. In deze periode heeft zij o.a. een kortdurende uitstap gemaakt naar de afdeling Pathologie van het Academisch Medisch Centrum te Amsterdam. In 1990 startte zij met de opleiding Pathologie aan de Erasmus Universiteit te Rotterdam (opleider Prof.dr. F.T. Bosman, later opgevolgd door Prof.dr. W.J. Mooi). In deze periode volgde zij perifere stages in het Diagnostisch Centrum SSDZ te Delft (opleider dr. C.A. Seldenrijk) en bij de Stichting Pathan, gelegen in het St. Franciscus Gasthuis, te Rotterdam (opleider dr. A.C. Jobsis).
Op 1 oktober 1995 werd zij geregistreerd als patholoog. Vanaf die datum tot oktober 1996 was zij chef de clinique Pathologie aan de Erasmus Universiteit te Rotterdam.

Vanaf 1 oktober 1996 is zij werkzaam als patholoog in het Canisius-Wilhelmina Ziekenhuis te Nijmegen. Sedert maart 2006 heeft zij de functie van integraal manager van de afdeling Pathologie in dit ziekenhuis op zich genomen en sedert oktober 2007 is zij opleider voor de B-opleiding binnen het opleidingscluster Pathologie van het UMC St.Radboud.

Carla Wauters is getrouwd met Marcel van Bentum, samen hebben zij een zoon: Pieter (geboren in 1999).

Publications and presentations

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32th annual San Antonio Breast Cancer Symposium San Antonio, Texas, USA

Contribution of CSF cytology to the management of breast cancer patients with neurological symptoms; a retrospective analysis over two decades
7th European Breast Cancer Conference March 2010, Barcelona, Spain